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Effects of Photoperiod, Temperature, and Diet on the Reconditioning Response, Blood Chemistry, and Gonad Maturation of Atlantic Salmon Kelts (*Salmo salar*) Held in Freshwater

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Johnston, C. E., R. W. Gray, A. McLennan, and A. Paterson. 1987. Effects of photoperiod, temperature, and diet on the reconditioning response, blood chemistry, and gonad maturation of Atlantic salmon kelts (*Salmo salar*) held in freshwater. Can. J. Fish. Aquat. Sci. 44: 702–711.

Changes in feeding behavior, rate of reconditioning, gonadal development, and maturation were investigated together with changes in plasma electrolytes, triglycerides, cholesterol, protein, and alkaline phosphatase in female Atlantic salmon kelts (*Salmo salar*) exposed to advanced and simulated natural photoperiods and warmer and natural water temperatures. Natural photoperiod cycles at natural and 12°C temperatures were most stimulatory for feeding behavior, reconditioning, gonadal development, and maturation. Advancing photoperiod only slightly stimulated gametogenesis at 12°C whereas at low temperature, vitellogenesis did not proceed and oocyte maturation did not occur. In all regimes, early reconditioning was associated with gonadal maturation. The levels of calcium, triglycerides, and alkaline phosphatase were markedly elevated in spawners and were coincident with temperature and photoperiod manipulation. No similar change occurred in nonspawners. Ovulation occurred earliest in the advanced photoperiod and 12°C temperature regime, suggesting an effect of both temperature and photoperiod on the rate of gonadal maturation. The factors influencing egg production and maturation in Atlantic salmon kelts are also discussed.

Les auteurs ont étudié les variations du comportement d'alimentation, du taux de rétablissement, du développement gonadique et de la maturation par rapport aux variations des teneurs plasmatiques en électrolytes, triglycérides, cholestérol, protéines et phosphatase alcaline chez des femelles de saumon atlantique (*Salmo salar*) de post-frai exposées à des photopériodes naturelles avancées et simulées ainsi qu'à des températures plus chaudes et naturelles. Les cycles de photopériode naturels aux températures naturelles et à 12°C se sont avérés des plus stimulants pour le comportement d'alimentation, le rétablissement, le développement gonadique et la maturation. Le fait de hâter la photopériode n'a que légèrement stimulé la gamétogénèse à 12°C et, aux températures inférieures, la vitellogénèse et la maturation des oocytes ne se sont pas produites. Sous tous les régimes, le rétablissement hâtif était lié à la maturation gonadique. Les teneurs en calcium, triglycérides et phosphatase alcaline étaient fortement élevées chez les poissons en frai et coïncidaient avec la modification de la température et de la photopériode. Aucune variation du même genre n'a été notée chez les poissons ne frayant pas. L'ovulation la plus hâtive a été notée en régime de photopériode avancée à 12°C; ce qui porte à croire à un effet de la température et de la photopériode sur le taux de maturation gonadique. Les auteurs traitent aussi des facteurs influant sur la production d'oeufs et la maturation chez les saumons atlantiques de post-frai.

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Many environmental and internal factors are thought to act as cues for the initiation of complex behavioral, physiological, biochemical, and neuroendocrine changes controlling the postspawning process of feeding, reconditioning, gonad maturation, and ovulation of salmonids (de Vlaming 1972, 1974; Billard and Breton 1979; Peter 1981; Saunders 1981; Bromage et al. 1982; Lam 1982). Among the most important environmental cues for salmonids are photoperiod and temperature (Henderson 1963; Billard et al. 1978; Whitehead et al. 1978; Peter and Crim 1979; Crim 1982). Other factors are salinity and nutritional status. Except for the report of Ducharme (1972), no other studies have been published regarding the effects of altering environmental conditions on the initiation and control of postspawning feeding behavior and reproduction in Atlantic salmon kelts (*Salmo salar*). The present study investigates the effects of four temperature and photoperiod regimes on feeding response, rate of reconditioning, hematology, blood plasma chemistry, and egg production in Atlantic salmon kelts artificially reared in freshwater.

Materials and Methods

Experimental Stock

Sixty-eight early-run Atlantic salmon kelts from the Miramichi River system, New Brunswick, were spawned at the Miramichi Fish Culture Station and transported to the Cardigan Fish Culture Station in Prince Edward Island between 28 and 30 November 1984.

Sixty-one kelts were females and seven were males. Based on scale readings, most were spawning for the first time; only eight females and four males were multiple spawners.

TABLE 1. Mineral and vitamin levels injected into each silverside prior to feeding.

Item	mg·silverside^{-1}
Minerals	
Sodium chloride	15.00
Potassium iodide	0.06
Maganous sulfate	1.40
Copper sulfate	0.35
Zinc sulfate	2.64
Magnesium sulfate	4.86
Water-soluble vitamins	
Thiamine	0.03
Riboflavin	0.06
Pyridoxine	0.03
Pantothenic acid	0.12
Biotin	0.003
Vitamin B_{12}	0.00006
Niacin	0.0004
Ascorbic acid	0.30
Folic acid	0.02
Inositol	1.20
Fat-soluble vitamins	
Vitamin A	40 IU
Vitamin D_3	4 IU
Vitamin E	40 IU
Vitamin K	0.18
Carophyll Red (10%)	0.30

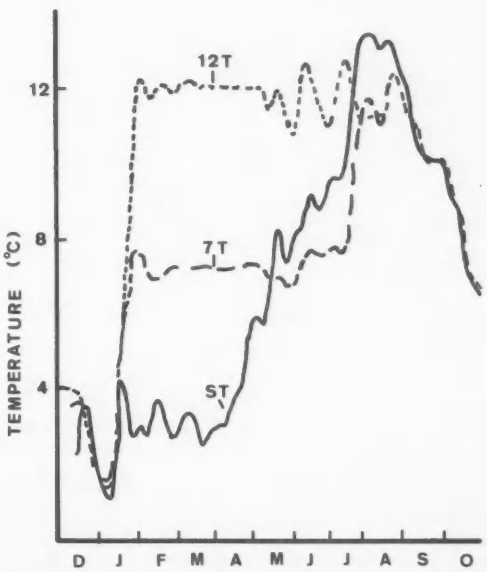

FIG. 1. Temperature regimes associated with advanced and simulated natural photoperiod regimes between December 1984 and November 1985.

Facilities and Experimental Conditions

Kelts were held indoors at the Cardigan Fish Culture Station is four 3-m-square, 1-m-deep fiberglass Swede tanks. Each tank was enclosed on top by a 1.1-m-high wall of masonite board that was lined with polyurethane foam carpet underlay. The lining was to prevent injury to jumping salmon when striking the wall. The top of the tank was made light-proof with 10-mil (0.254-mm) black polyethylene sheeting. Each tank was illuminated with four 40-W General Electric cool white fluorescent light tubes placed 1.2 m above the water surface and covered with plastic light diffusers. An average light intensity of 1076 lx was recorded at the water surface in the central area of the tank. The length of the photoperiod in the simulated natural photoperiod regime was controlled by a photocell situated outdoors. Light and dark periods corresponded with those outside; no attempt was made to reproduce

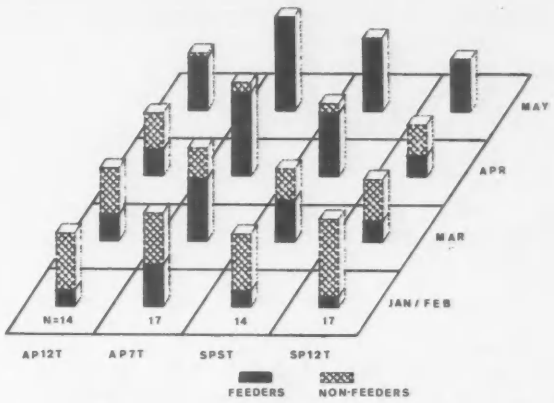

FIG. 2. Number of feeders to nonfeeders in each experimental regime between January and May 1985.

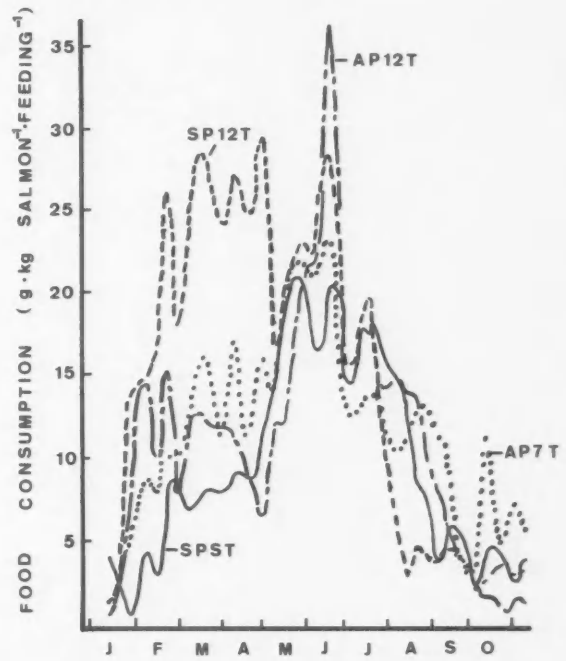

FIG. 3. Food consumption in experimental regimes SPST, SP12T, AP12T, and AP7T between January and November 1985.

natural intensities, wavelengths, incidences of light, or dawn and dusk. In tanks with advanced photoperiod regimes, the lengths of the light and dark periods were manually set on timers connected to the lights.

Each tank was supplied with well water flowing at a rate of 38 L·min^{-1}. Water passing into each tank was degassed and equilibrated with atmospheric gases by falling over a vertical column of plastic rings. Additional aeration was achieved by pumping air through 7.3 m of porous plastic tubing arranged peripherally around the bottom of the tank. Water depth in each tank was 56 cm. The temperature of the water was controlled by heating or cooling the 7°C well water. Water was heated to 12 ± 1°C using an oil-fired furnace and heat exchanger and aerated as previously described. Water was cooled in the winter in the simulated natural temperature regime using a specially designed cooling system of epoxy-coated copper tubes. The tubes were situated outdoors in a raceway filled with continuously flowing river water. Well water of 7°C was passed through the cooling tubes, in a countercurrent flow to the river water, partially cooled, and then passed to a reservoir tank containing three Mino-cool refrigeration units for final cooling before being gravity fed to each culture tank.

When the kelts arrived at the Cardigan Fish Culture Station, they were randomly distributed among the four tanks at 17 salmon per tank. During December, all kelts were held in river water, natural photoperiod, and without food.

Commencing on 1 January 1985 each group of kelts was placed on one of four different temperature and photoperiod regimes:

Experimental group	Experimental protocol
SPST	This group received a simulated natural photoperiod and simulated natural seasonal temperatures (see Fig. 1)
SP12T	This group received a simulated natural photoperiod and water temperature that increased beginning 1 January at 1°C·d^{-1} until 12°C. This temperature was maintained until September when it declined in phase with the Cardigan River water temperature
AP12T	This group received an advanced photoperiod in which the length of the light period was increased from 10 h in January to 15.5 h in March. This photoperiod was continued until 21 June and declined naturally thereafter. Water temperature followed that described for SP12T
AP7T	This group received an advanced photoperiod as described for AP12T. Water temperature increased beginning 1 January at 1°C·d^{-1} until 7°C. This temperature was maintained until late July when it was increased to 12°C until September and thereafter declined naturally

Silversides (*Menidia menidia*) caught in the fall and frozen at −20°C were used as food during the experimental period.

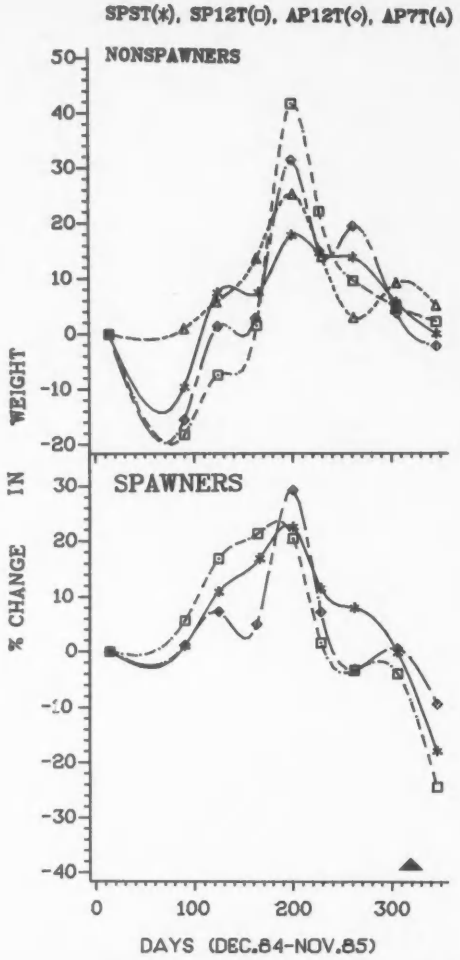

FIG. 4. Percentage weight gain or loss by nonspawners and spawners in each experimental regime between December 1984 and November 1985. The arrowhead represents the period of stripping for spawners.

Prior to feeding, silversides were thawed, injected with mineral and vitamin mixtures (Table 1), and then thrown individually near the mouth of each salmon twice a day. In late May because of the large number of silversides consumed daily, only one half of them were injected with minerals and vitamins. In late July, carophyll red was injected into the silversides as a nutritional supplement and to add color to the developing eggs. Salmon were fed to repletion at each feeding, 5 d each week. The amount of food consumed by each salmon was recorded together with observations on feeding behavior. The efficiency of food conversion to body weight (change in body weight in grams/food consumed in grams × 100) was calculated at intervals throughout the study period.

Growth Measurements

Starting on 14 December, weight and length measurements were made at approximately monthly intervals. Prior to each weighing, salmon were starved for 24 h. All salmon were

TABLE 2. Weight-conversion efficiencies for Atlantic salmon nonspawners (NS) and spawners (S) in each experimental regime for each interval between December 1984 and November 1985.

Time interval	Conversion efficiency (%)						
	SPST		SP12T		AP12T		AP7T
	NS	S	NS	S	NS	S	NS
14 Dec. 1984 – 1 Mar. 1985	39.1	9.8	—	12.1	4.2	3.1	—
2 Mar. – 4 Apr. 1985	48.9	41.0	—	23.5	13.2	17.6	2.0
5 Apr. – 13 May 1985	46.9	31.0	19.5	23.1	12.9	16.7	22.8
14 May – 20 June 1985	17.1	23.8	22.5	22.9	24.6	33.2	24.6
21 June – 17 July 1985	7.4	24.0	23.1	9.1	23.1	21.9	30.4
18 July – 20 Aug. 1985	3.5	23.2	16.5	—	27.5	—	6.1
21 Aug. – 3 Oct. 1985	14.9	—	7.3	—	—	—	6.2
4 Oct. – 10 Nov. 1985	—	—	—	—	—	—	—

anaesthetized with MS222 (0.1 $g \cdot L^{-1}$), weighed, measured for fork length, and tagged with numbered 2.54-cm Peterson disc tags. Condition factor was determined using weight (in grams)/fork length^3 (in cm) × 100. The percentage change in fresh weight (gain or loss) for each monthly interval was determined for spawners and nonspawners by dividing the change in weight during the interval by the weight of the salmon at the beginning of the interval multiplied by 100.

Hematology and Blood Plasma Chemistry

Blood was collected monthly from a sample of four female kelts from each regime. A 1- to 2-mL blood sample was taken by direct cardiac puncture into lithium heparinized syringes. (Hille (1982) suggested that these methods induce the least possible change in most blood parameters.) Blood samples were immediately divided into two aliquots, one for hematological measurements and the other for blood plasma chemistry. Triplicate hematocrit and leucocrit measurements were made immediately by drawing well-mixed samples of blood into heparinized capillary tubes (1.5 × 75 mm) and centrifuging at 8000 rpm for 4 min. Leucocrit measurements were made from the buffy coat zone using a microscope at a magnification of 15×. Total hemoglobin was determined using the cyanomethemoglobin method using Drabkin's reagent and a stable human hemoglobin standard (Sigma Tech. Bull. 525, Sigma Chemical Co., St. Louis, MO). Blood plasma was separated after centrifugation and stored at 0–2°C until assays could be performed. Plasma Na^+ and K^+ levels were measured using a Nova-1 specific ion analyzer. Plasma chloride concentrations were measured using a Buchler digital chloridometer. Plasma Mg^{2+}, Ca^{2+}, total protein, triglycerides, cholesterol, and alkaline phosphatase were measured using assay kits prepared by Diagnostic Chemicals Ltd., Charlottetown, P.E.I. Each sample was assayed in duplicate or triplicate depending upon available plasma volume.

Egg Measurements

Reconditioned kelts were spawned during October and the resulting eggs were fertilized, water hardened for 3 h, disinfected with a 1% solution of polyvinyl-pyrrolidone-iodine for 10 min, rinsed for 20 min, and then laid down. Egg number was determined by the displacement method (Burrows 1951), and after water hardening, egg diameters were measured in a 15-cm egg trough (von Bayer 1910).

Differences between means of the parameters measured were tested statistically by either a Student's *t*-test or a Duncan's multiple range test.

Results

Beginning in January a small number of fish from each group began feeding the first day food was offered. By the end of the month, 21 kelts in all four regimes had initiated feeding; 14 were from the low-temperature SPST and AP7T regimes. In the SPST regime, seven kelts initiated feeding when water temperatures were between 2 and 4°C. Between 30 and 50% of the feeders in each regime were those that had been sampled for blood. Removal of 1–2 mL of blood may have stimulated a stronger feeding response by altering the osmotic or ionic composition of the blood following sampling. In all groups, feeding fish became very aggressive in the presence of food and a heirarchy of dominant and subdominant individuals developed. Dominant kelts tended to intimidate subdominant fish during feeding, and only after dominant fish were satiated was it possible to stimulate subdominant feeding. Some kelts would only eat when the silverside was thrown close to their mouth. Rarely would kelts take silversides from the bottom of the tank. In some cases, failure to feed was not directly related to an inability to initiate a feeding response, but rather to an inability to swallow food. Sometimes a kelt would take a silverside into its mouth, begin swallowing, and then regurgitate it. Swallowing food appeared to irritate either the pharynx or upper esophagus. Since the digestive system degenerates prior to and during spawning, this regurgitation response may have been caused by a swollen and sensitive esophagus. After swallowing food several times, the food rejection response was less prevalent.

The ratio of feeders and nonfeeders in each regime varied (Fig. 2). The number of feeders increased gradually between January and May, and by May, most salmon in each regime had initiated feeding. Any salmon that had not done so by late May was artificially stimulated to feed by repeatedly placing silversides in its mouth until one was swallowed. Once food was accepted and swallowed, the feeding response became stronger and more voluntary. A comparison of the feeding responses in each regime revealed that spawners were always the first to feed and initiated feeding in January or February.

Food consumption (grams of silverside consumed per kilogram of salmon per feeding) varied for the different regimes

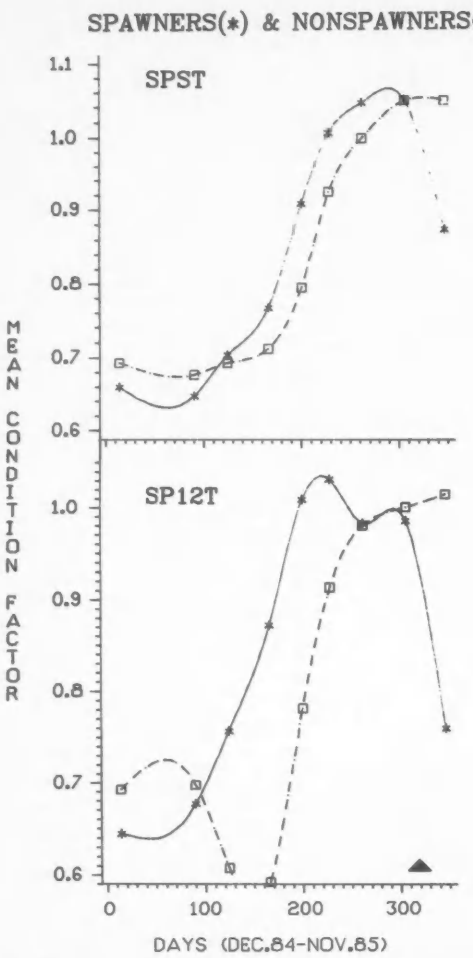

FIG. 5. Mean condition factors for nonspawners and spawners in SPST and SP12T between December 1984 and November 1985. The arrowhead represents the period of stripping for spawners.

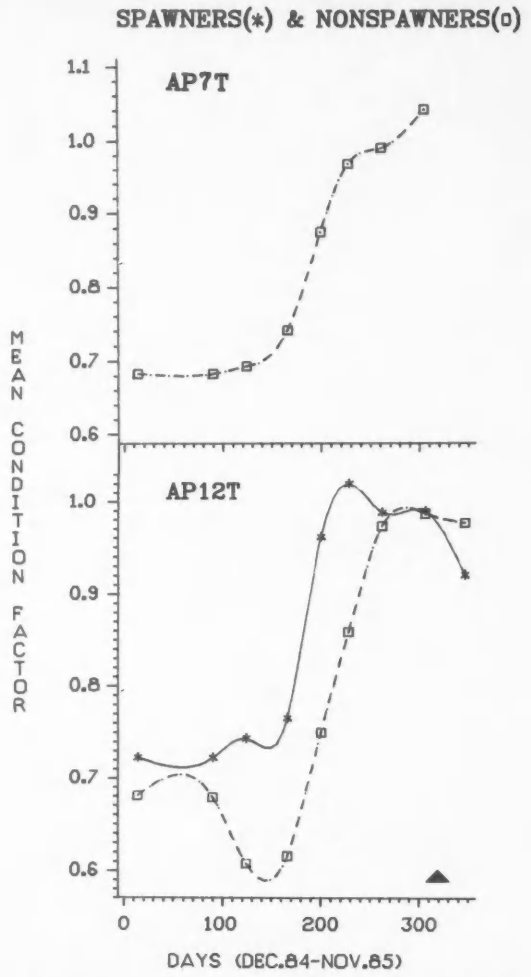

Fig. 6. Mean condition factors for nonspawners and spawners in AP7T and AP12T between December 1984 and November 1985. The arrowhead represents the period of stripping for spawners.

during the experimental period (Fig. 3). Between February and May, SP12T stimulated the greatest food consumption (15–28 $g \cdot kg^{-1} \cdot feeding^{-1}$); SPST resulted in the lowest (3–12 $g \cdot kg^{-1} \cdot feeding^{-1}$) and the other two regimes were intermediate, varying between 6 and 15 $g \cdot kg^{-1} \cdot feeding^{-1}$. The slight weekly oscillations in feeding levels that occurred may have been associated with weighing, sampling, or salt baths. Between May and June there was a marked increase in food consumption in most regimes, apparently associated with an endogenous feeding rhythm that was independent of temperature and photoperiod. Between June and July, salmon in AP12T that did not eat as well between December and May surpassed the food consumption of salmon in the other regimes and attained a record high of 35 $g \cdot kg^{-1} \cdot feeding^{-1}$. From late July to October, food consumption levels declined in all regimes with the greatest declines occurring in SP12T and AP12T. These declines were directly related to cooler water temperature and to reproductive maturation.

Following spawning in October, we divided the fish into two groups, those that matured (spawners) and those that did not mature (nonspawners), and compared the parameters measured. A comparison of the percentage gain or loss in fresh weight for spawners and nonspawners in each regime demonstrated clearly that between January and mid-May, there were earlier and significantly greater ($p < 0.05$) gains in body weight for spawners than nonspawners (Fig. 4). In the 12T regimes, nonspawners failed to initiate early feeding and lost body weight and condition factor. In contrast, kelts in the low-temperature conditions (ST and 7T) that failed to feed did not lose as much weight nor did they take as long to recover the weight lost. In all regimes, both spawners and nonspawners showed a peak gain in body weight between May and June corresponding to the peak in feeding activity.

The efficiencies of converting food into body weight by spawners and nonspawners are shown in Table 2. The highest

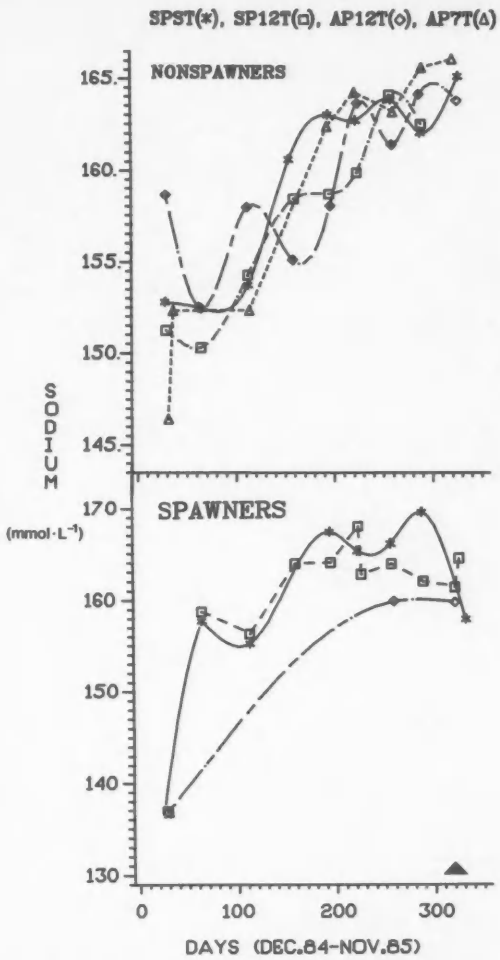

FIG. 7. Mean plasma sodium levels for nonspawners and spawners in the experimental regimes between December 1984 and November 1985. Each point represents the mean of one to four kelts. The arrowhead represents the period of stripping for spawners.

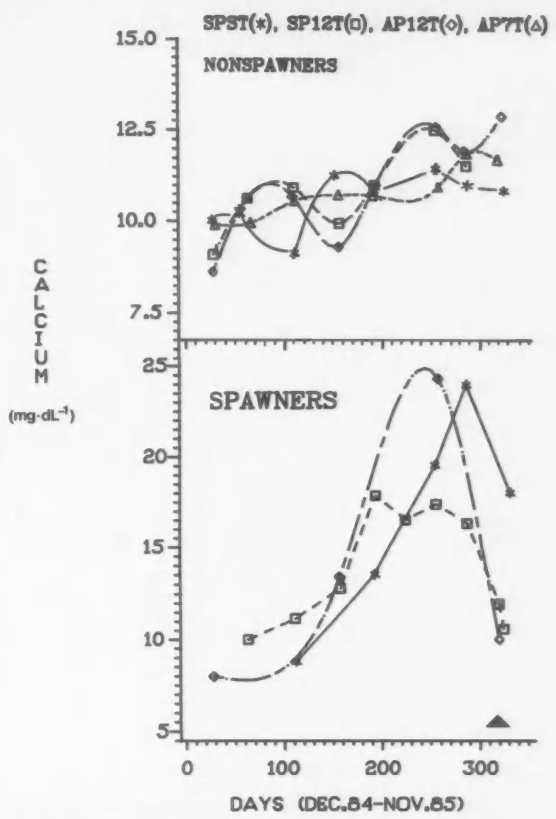

FIG. 8. Mean plasma calcium levels for nonspawners and spawners in the experimental regimes between December 1984 and November 1985. Each point represents the mean of one to four kelts. The arrowhead represents the period of stripping for spawners.

conversion efficiencies were in the natural photoperiod conditions and were temperature dependent. Advancing the photoperiod to summer conditions in March initially resulted in very low conversion efficiencies, but these efficiencies increased as salmon adjusted to the conditions.

Significantly higher condition factors were observed for spawners between March (90 d) and June (210 d) than nonspawners in each regime (Fig. 5 and 6). More marked differences in condition factor were evident at 12°C than at lower temperatures. By late July (240 d), condition factor among groups in each regime was not significantly different. Following spawning, condition factor declined but remained well above the level found at the beginning of the experiment.

Kelt mortalities during December to May varied with regime, and the largest number of mortalities occurred in the high-temperature regimes AP12T and SP12T. In the 12T regimes, 2 of 12 kelts which lost weight and died were males. In the SPST regime, only three kelts died, two of which were males. There were no mortalities in AP7T. Most mortalities occurred as a result of marked weight loss at 12°C and fungal infections by *Saprolegnia* and gill parasites (*Salmincola salmoneus*). *Salmincola* was controlled by removing adults from the gills each month whereas fungal infections were prevented by exposing the kelts to a salt treatment of 20‰ for 5–6 h every 20–30 d.

Salmincola infestations may have influenced kelt feeding and/or health particularly in the AP12T regime. Between December and February the number of *Salmincola* per infected fish ranged between 79 and 154 with most salmon being infected (McGladdery and Johnston 1987). In February, salmon in 12T regimes had approximately twice as many *Salmincola* per infected fish as salmon in ST or 7T regimes. Removal of adult *Salmincola* from the gills resulted in 22–65 *Salmincola*·fish^{-1} in March and only 1–14 in June. Kelts in AP12T always had a slightly higher level of infection than those of the other regimes. This may have influenced early weight gains and condition.

Salmincola infestations and secondary infections by *Saprolegnia* caused leucocrit and white blood cell counts to rise from 1.1 to 1.6 and 8300 to 30 900 cells·mm^{-3}, respectively, be-

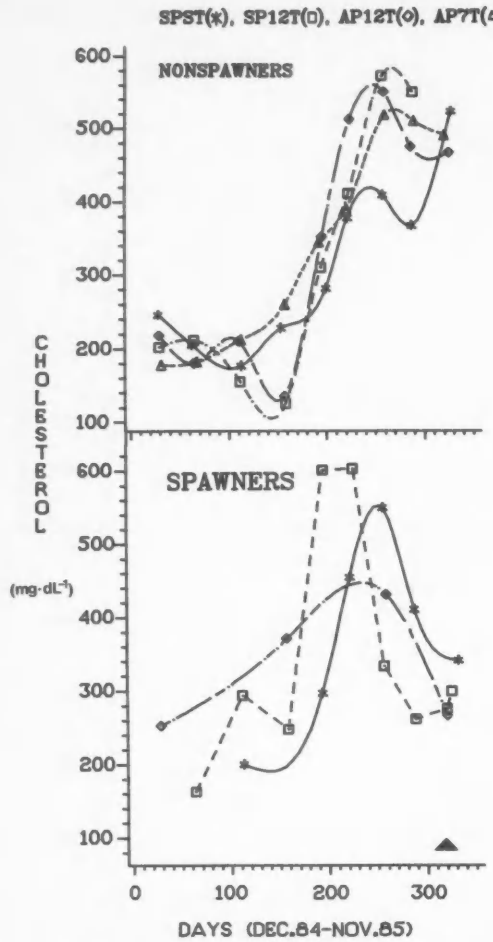

FIG. 9. Mean plasma cholesterol levels for nonspawners and spawners in the experimental regimes between December 1984 and November 1985. Each point represents the mean of one to four kelts. The arrowhead represents the period of stripping for spawners.

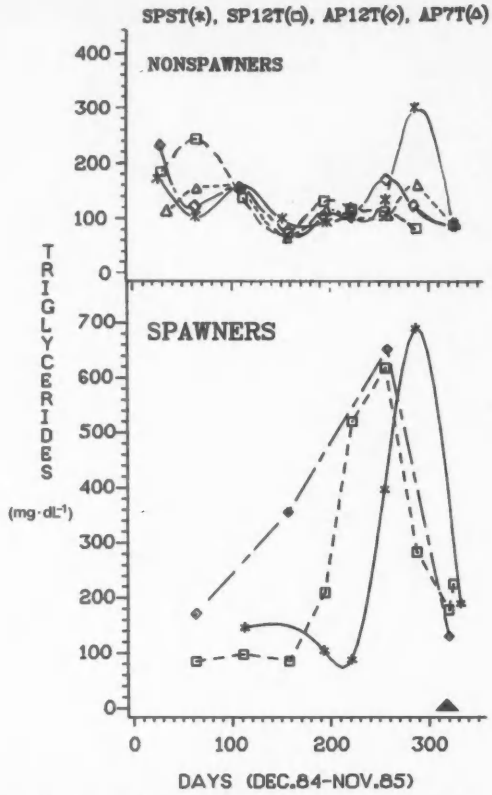

FIG. 10. Mean plasma triglyceride levels for nonspawners and spawners in the experimental regimes between December 1984 and November 1985. Each point represents the mean of one to four kelts. The arrowhead represents the period of stripping for spawners.

tween December and April. White blood cell counts were not significantly different between regimes or between spawners and nonspawners. During the same period, hematocrit, hemoglobin, and red blood cell counts varied between 23.5 and 39.0%, 6.2 and 13.7 $g \cdot dL^{-1}$, and 0.630×10^6 and 1.12×10^6 red blood cells $\cdot mm^{-3}$, respectively. No marked differences in the levels of these parameters were observed between spawners and nonspawners. The extent to which changes in hematological parameters influenced early reconditioning, gametogenesis, or vitellogenesis is not clear. The data suggest that this was not an important factor.

Blood chemistry parameters were monitored monthly to evaluate both the health status and the cyclical changes associated with nutrition, gametogenesis, vitellogenesis, and ovulation. Dietary supplements of minerals significantly elevated plasma Na^+, Ca^{2+}, and Mg^{2+} levels in both nonspawners and spawners during the experimental period (Fig. 7 and 8). Significantly higher ($p < 0.05$) levels of Ca^{2+} were observed in spawners than nonspawners prior to spawning in all regimes (Fig. 8). Ca^{2+} levels rose in late June and early July in spawners from the 12T regimes and only later in those from the ST regime. Shortly after ovulation there was a marked decline in Ca^{2+} levels. Increases in Ca^{2+} were coincident with increases in triglyceride and alkaline phosphatase. The Mg^{2+} levels in spawners rose above normal between August and October, but only in SPST were the increases significant. There was no consistent pattern of elevation in plasma K^+ and Cl^- levels in spawners prior to ovulation. There was a significant rise ($p < 0.05$) in plasma K^+ levels in both groups during periods of intense feeding and these declined as food consumption fell off. A significant ($p < 0.05$) decline in K^+ levels occurred in spawners from SPST and SP12T following ovulation. Plasma Cl^- levels increased with food consumption and remained unchanged thereafter.

Plasma cholesterol levels in both spawners and nonspawners increased significantly during the experimental period in all regimes (Fig. 9). Increases were associated with food consumption. Nonspawners maintained high cholesterol levels during October and November whereas spawners showed marked declines ($p < 0.05$) in the levels. Spawners had significantly higher cholesterol levels in 12T than in ST in June and July, but this relationship was reversed in August and September.

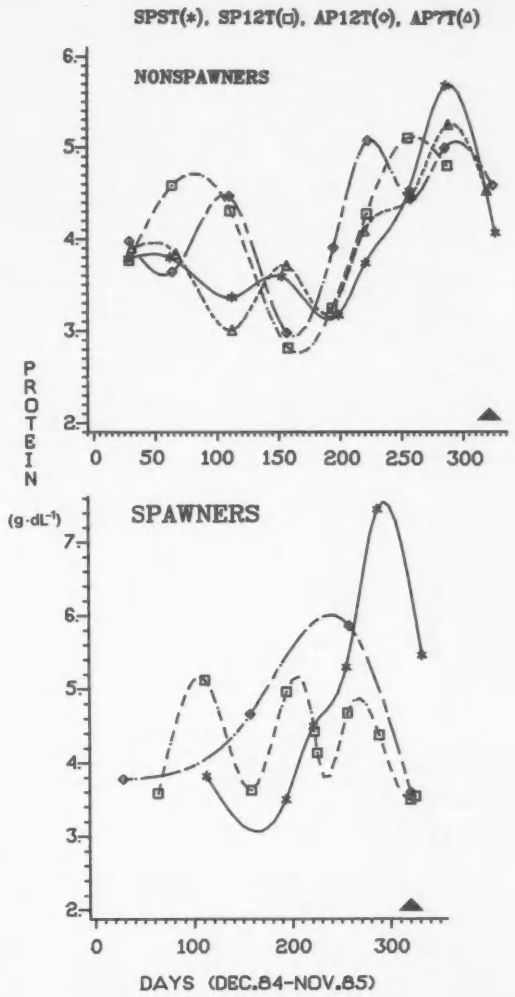

FIG. 11. Mean plasma protein levels for nonspawners and spawners in the experimental regimes between December 1984 and November 1985. Each point represents the mean of one to four kelts. The arrowhead represents the period of stripping for spawners.

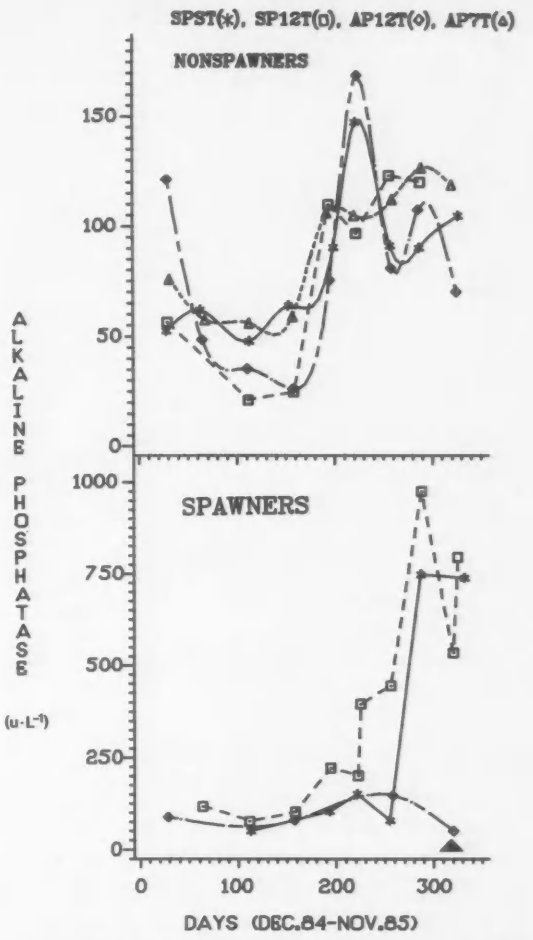

FIG. 12. Mean plasma alkaline phosphatase activity for nonspawners and spawners in the experimental regimes between December 1984 and November 1985. Each point represents the mean of one to four kelts. The arrowhead represents the period of stripping for spawners.

Triglyceride levels for nonspawners fluctuated primarily between 80 and 200 mg·dL^{-1} and showed no correlation with season or with food consumption (Fig. 10). However, triglyceride levels for spawners increased significantly to over 600 mg·dL^{-1} in the 12T regimes during July and August and in the SPST regime in September. The response came earlier in the AP12T regime. Declines in triglyceride levels coincided with vitellogenesis.

Plasma protein level increases in both spawners and nonspawners correlated with the level of parasitism and with food consumption (Fig. 11). There was a rise in protein levels in some spawners during oocyte maturation, but the trend was not evident in all regimes. Following ovulation, plasma protein levels tended to decline.

Plasma alkaline phosphatase (AP) levels in nonspawners varied between 25 and 170 units (u)·L^{-1}, and elevations were positively correlated with food consumption (Fig. 12). AP activity in spawners from SP12T and SPST regimes was markedly increased (600–900 u·L^{-1}) during vitellogenesis and was significantly higher than in nonspawners. AP appears to be an important enzyme system providing a source of phosphate for synthesis of vitellogenin by the liver. AP levels in the AP12T spawners were markedly lower than the other regimes.

Data for spawning time, fecundity, and egg size from the different regimes suggest that normal photoperiod and warm temperature were necessary for gonadal maturation (Table 3). Although only one salmon matured in AP12T, the date of ovulation was 8–14 d before those in the SP12T and 21 d before those in SPST. SPST salmon ovulated at exactly the same time as wild salmon held in the hatchery from the Morell River, P.E.I. In AP12T the number of eggs produced per centimetre fork length was low (37.4) and eggs were irregular in shape, but not different in diameter, whereas in SP12T and SPST the mean number was 75.2 and 103.4, respectively, and

TABLE 3. Fecundity of Atlantic salmon kelts reared under different photoperiod and temperature conditions, 1984–85.

Rearing conditions	Date spawned	Fork length (cm)	Egg diameter (cm)	Number of eggs	% maturing
AP7T	—	—	—	—	0
AP12T	7 Oct.	74.0	0.61	2 768	10
SPST	28, 29, 30 Oct., 10 Nov.	77.4	0.52	11 274	
	28 Oct.	78.3	0.59	5 760	25
	28 Oct.	74.0	0.57	6 788	
SP12T	15 Oct.	72.5	0.63	5 104	
	17 Oct.	68.9	0.62	4 506	40
	17 Oct	71.4	0.61	6 363	
	21 Oct.	66.5	0.59	5 042	

eggs were normal. There was no significant difference between SP12T and SPST in the number of eggs per centimetre fork length. There was no significant difference in egg size among the regimes. Forty percent of the females matured in the SP12T whereas 25, 10, and 0% matured in SPST, AP12T, and AP7T, respectively.

Discussion

In the present study, natural photoperiod cycles combined with 12°C or natural temperature conditions stimulated oogenesis and vitellogenesis more than the other photoperiod and temperature regimes. Advancing photoperiod had only a slight stimulating effect at high temperature and an inhibiting effect at low temperature. Although oogenesis occurred at low temperature, vitellogenesis did not proceed normally as evidenced by the size of ovaries taken from a kelt in the AP7T regime in early November 1985 and in the blood chemistry data. In this case, ovaries were 16 cm long and 2–3 cm wide with oocytes approximately 1 mm in diameter; plasma Ca^{2+}, triglycerides, and AP levels did not rise substantially. Korsgaard et al. (1986) observed that a vitellogenic response to estradiol treatment could not be elicited at 3°C but could be elicited at 10°C in female salmon postsmolts. Advancing temperature to 12°C in March also appears to have had an inhibiting effect on gonad development. Gillet et al. (1978) have shown that GtH secretion is suppressed in goldfish (*Carassius auratus*) when they are exposed to high temperature during fall or winter when oogenesis is occurring. The failure of kelts to spawn in AP12T may be due to a similar temperature effect on GtH release.

Oogenesis and vitellogenesis proceeded in kelts that had restored body protein and fat early in the spring. Kelts that were slow to restore energy reserves did not spawn although oogenesis was initiated. All kelts that died during the experimental period in the different regimes showed some gonadal development. For example, by June or July, gonads from kelts in SPST were 15–16 and 1.5–2.0 cm in length and width, respectively, with oocyte sizes of 0.1–1.0 mm. These observations suggest that increasing photoperiod is essential for the initiation of gonadal growth and support the recent findings of Whitehead and Bromage (1980) and Scott and Sumpter (1983) for rainbow trout (*Salmo gairdneri*). These data also suggest that ovarian development begins but becomes arrested through the secondary growth phase of exogenous vitellogenesis. Although the underlying reasons for this arrest are not completely clear, it most probably involves a suppression of the synthesis or release of either the gonadotropin releasing factors from the hypothalamus, GtH from the pituitary, and/or estrogen from the ovary. In studies on other salmonids, plasma GtH levels have been shown to increase above 10 $ng \cdot mL^{-1}$ during vitellogenesis and final oocyte maturation (Billard et al. 1978; Crim and Idler 1978; Crim et al. 1983). It is possible that hypothalamic releasing factors fail to stimulate the release of sufficient levels of GtH for the maintenance of estrogen production. Alternatively, it may be that estrogen synthesis and/or release by the thecal and/or granulosa cells of the ovary is/are insufficient or impaired. Without estrogen and some GtH, vitellogenesis will not proceed (Whitehead et al. 1978; Upadhyay et al. 1978; Bromage et al. 1982). Estrogen has been shown to significantly increase the synthesis of vitellogenin by the liver and elevate plasma Ca^{2+}, Mg^{2+}, protein, and triglyceride (Redshaw et al. 1969; Korsgaard and Petersen 1979; offset this physiological deficiency by the application of testosterone, estrogen, and/or some GtH during the period of vitellogenesis to stimulate further oocyte development and maturation.

Ca^{2+} and AP data for some nonspawners in July and August indicated that levels may rise and later decline to lower levels. This suggests that some ovarian growth may take place and then stop. The factors controlling this event are not immediately clear from the data. It is possible that stress created by the anesthetic or blood sampling procedures may have influenced GtH release and final oocyte maturation.

The length of the refractory period following spawning may be genetically determined through endogenous rhythms. Some strains of kelts may reproduce in consecutive years whereas others may reproduce in alternate years (Gardner 1976; Saunders 1981; Saunders et al. 1983; Jessop 1986). The degree to which environmental cues can override these endogenously determined rhythms is still unknown. It is apparent from the data that there is a clearly defined endogenous feeding rhythm that is to some extent independent of temperature and photoperiod. Further studies will have to be undertaken to clarify the environmental cues that can override such endogenous cycles and the factors that suppress and stimulate oogenesis and vitellogenesis.

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Evidence for Size-Selective Mortality of Juvenile Sockeye Salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia

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West, C. J., and P. A. Larkin. 1987. Evidence for size-selective mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia. Can. J. Fish. Aquat. Sci. 44: 712–721.

Otolith – body length relations and back-calculation procedures were used to test the hypothesis that mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia, is size selective. Samples of the 1978 brood of sockeye were collected as fry from spawning tributaries as juveniles in the main basin, and as smolts at the outlet. Total otolith length was chosen as the most useful otolith dimension for back-calculation of fork length at emergence. Sockeye from the various tributaries show different fork length – otolith length relationships necessitating a weighting procedure for comparisons involving samples of mixed stocks from the lake. Instantaneous daily growth rate and ln fork length during the early lake-rearing period were significantly correlated. Smaller juveniles grew more slowly than larger individuals in mid-July, and a hierarchy of sizes was maintained. The distributions of total otolith length at emergence for fry, surviving juveniles, and smolts indicate selective mortality of fish with smaller otoliths, hence of small size at emergence. Survivals from the lower and upper halves of the initial total otolith length distribution were 8.9 and 63.6%, respectively. Comparable estimates for back-calculated fork lengths at emergence were 27.2 and 43.4%. Size-selective mortality is most intense in the late summer and early autumn, and may be associated with predation and parasitism.

Les auteurs ont utilisé les relations de longueur entre les otolithes et le corps de même que des procédures de calcul à rebours pour tester l'hypothèse voulant que la mortalité des saumons nerka (*Oncorhynchus nerka*) juvéniles du lac Babine (Colombie-Britannique) soit sélective quant à la taille. Des saumons nerka de la classe annuelle de 1978 ont été prélevés sous forme d'alevins dans les tributaires de frai, de juvéniles dans le bassin principal et de smolts dans l'embouchure du cours d'eau. La longueur totale des otolithes a été choisie comme dimension la plus utile au calcul à rebours de la longueur à la fourche à l'émergence. Les saumons provenant de tributaires différents présentaient des relations longueur à la fourche – longueur des otolithes variables, ce qui a nécessité de peser les poissons afin de permettre la comparaison d'échantillons de stocks mixtes prélevés dans le lac. Le taux de croissance quotidien instantané et le logarithme de la longueur à la fourche présentaient une corrélation significative au début de la période de croissance en lac. Le taux de croissance des plus petits juvéniles était inférieur à celui des indidivus plus gros à la mi-juillet et une hiérarchie des tailles a été maintenue. Les distributions des longueurs totales des otolithes des alevins à l'émergence, des juvéniles ayant survécu et des smolts indiquaient l'existence d'une mortalité touchant sélectivement les poissons possédant les plus petits otolithes, donc les plus petits poissons à l'émergence. Les taux de survie des poissons correspondant aux demies inférieure et supérieure de la distribution des longueurs totales des otolithes de départ étaient, respectivement, de 8,9 et de 63,6 %. Des estimations comparables, calculées à rebours, des longueurs à la fourche à l'émergence donnaient des valeurs respectives de 27,2 et de 43,4 %. La mortalité sélective quant à la taille est plus intense à la fin de l'été et au début de l'automne et pourrait être liée à la prédation et au parasitisme.

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The purpose of this study was to ascertain the effect of body size at emergence on the subsequent survival of sockeye salmon (*Oncorhynchus nerka*) fry in Babine Lake, British Columbia. The early life history of juvenile sockeye has been studied intensively but most studies have provided only incidental information on size-selective mortality. Releases of marked juveniles in Cultus Lake (Foerster 1938) did not indicate a difference in the percentage return as adults from the sea of wild fish and the larger of reared individuals. Increased survival of the reared fish to the migrant smolt stage may have been offset by increased residualism. Predation on juvenile sockeye was also investigated in Cultus Lake (Ricker 1941; Foerster and Ricker 1941), and although increased predation on smaller individuals was hypothesized, differential mortality within year classes remained untested.

Boyce (1982) pursued a relationship between initial size of fry at lake entry and susceptibility to parasitic infection (Boyce 1974), but disease caused severe mortalities and vitiated comparison among the test groups. Dependence of juvenile sockeye survival on available food supply in Babine Lake was

considered by Rankin (1977) but the mechanism of mortality was not investigated.

A mark–release study by McDonald (1969) on the 1965 brood at Babine Lake revealed that although initial size differences between Fulton River and Fulton spawning channel fry persisted to the smolt stage, survival rates of the two groups were similar.

Thus, while size-dependent survival of juvenile sockeye has been considered for several stocks and associated with predation and parasitism, the mortality or its possible mechanisms have not been demonstrated.

The use of daily growth rings in sagitta otoliths for size determination has been well documented (e.g. Jonsson and Stenseth 1977; Marshall and Parker 1982; Neilson and Geen 1982; Struhsaker and Uchiyama 1976; Wild and Foreman 1980; Wilson and Larkin 1982). Otolith growth rings which correspond statistically to daily growth increments in juvenile sockeye (Wilson and Larkin 1982) provide a useful tool to reexamine the hypothesis: Do slow-growing individuals have a lower survival rate?

In this study, we have tested for size-selective mortality by examining differences between the length frequency distribution of the fry population which entered Babine Lake and that at emergence back-calculated from juveniles and smolts of the same brood captured at later dates.

Methods

Fish Collection and Preservation

Samples of the 1978 brood were collected as fry in the spring of 1979 in the spawning tributaries of the Babine Lake system, as juveniles in the summer of 1979 in the main basin of Babine Lake, and as smolts in the spring of 1980 at the time of seaward migration. Three times weekly at the peak time of the nightly migration from early May to mid-June, 50 fry samples were taken at each of five locations: Fulton River, Fulton spawning Channels 1 and 2, Pinkut Creek, and Pinkut spawning channel. After length and weight measurements of anaesthetized fry were taken, the fry were preserved in 70% ethanol (Brothers et al. 1983) for subsequent extraction of otoliths. No difficulties were encountered with the effect of 70% ethanol on otoliths, those of sockeye being less susceptible than those of larval cod (*Gadus morhua*), as reported by Radtke and Waiwood (1980). Estimates of the number of fry migrating nightly from each location were provided from the fry enumeration programs of the Department of Fisheries and Oceans. Nightly migration estimates were used to weight length frequency distributions by location from the fry samples.

Juveniles were captured with a 2 × 2 m midwater trawl (Gjernes 1979), towed by a single boat from a distance of 60 m, and fished at a depth of 2 m. A Furuno echo sounder aided selection of likely capture sites. During July 11–13, August 22–24, and September 19–21, trawling was conducted throughout the main basin, individual sampling locations collectively reflecting the seasonal distribution pattern of juveniles as previously reported by McDonald (1969).

Smolt samples were collected throughout the period of migration in late May and early June 1979 at the outlet of the Babine–Nilkitkwa Lake system where a smolt sampling program is carried out annually by the Department of Fisheries and Oceans. Fifty smolts taken randomly from every second night's catch were preserved in 70% ethanol. As with fry sampling, nightly estimates from the smolt enumeration program were used to weight the samples to calculate population length frequency distributions.

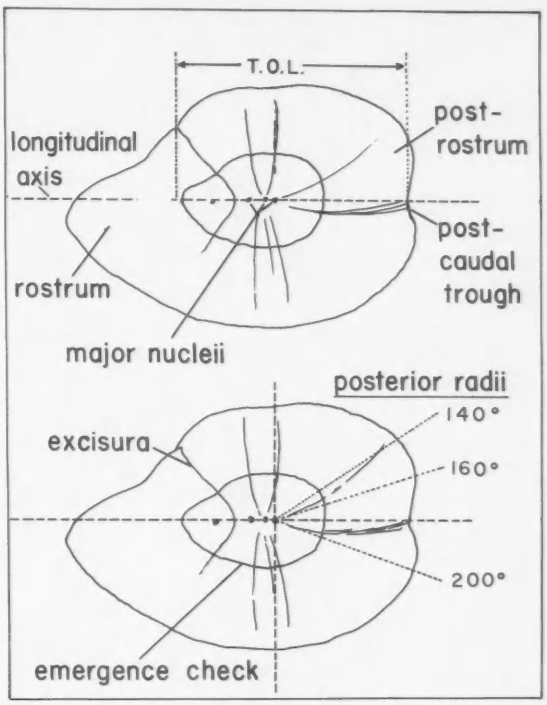

FIG. 1. Two diagrams of the sagitta of juvenile sockeye viewed from the distal side indicating various features used in orientation for measurement. T.O.L. = total otolith length.

Otolith Preparation and Measurement

Fish <40 mm were held in an inverted position, the opercular plates removed exposing the gill structures, and the otoliths then teased from the sacculus on the tip of a probe. For larger fish (40–90 mm) the head was split along the longitudinal axis exposing on either side the sagittae which could be removed with forceps. Otoliths were cleaned of membranes and stored in MicroTest 3034 tissue culture plates. Left or right otoliths were randomly selected for observation and measurement. Otoliths were either attached with an acrylic adhesive to microscope slides, sulcal surface upwards, and ground against sintered glass plate using carborundum No. 50 aluminum oxide powder mixed with water, or for larger fish, prepared by the method of Neilson and Geen (1982).

Prior to photographing, mounted otoliths were cleared in glycerol. The time required for rings to become and remain visible is related to otolith size. Fry otoliths exhibit maximum ring counts after approximately 6 h of clearing and begin to overclarify after another 6 h. The clearing process can be reversed by soaking in ethanol. Smolt otoliths require several days to clear, but remain readable for a much longer period. As a standard procedure before photographing, fry otoliths were cleared for 4–6 h, juvenile samples for 24 h, and smolt otoliths

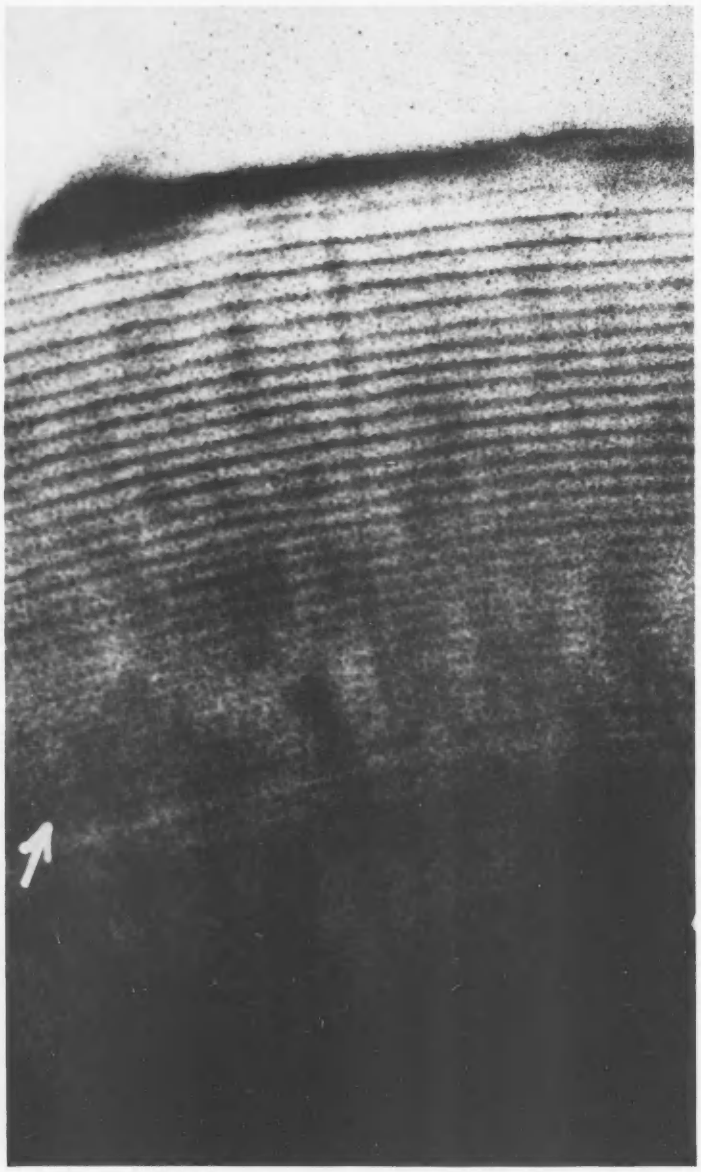

FIG. 2. Portion of sagitta of juvenile sockeye indicating the light zone bordered by a distinct dark band which indicates emergence and the transition to active foraging.

from 2 to 3 d.

The sagitta otoliths of sockeye fry are distinctive but their shape varies among individual fish, and those of a single fish may have different shapes. To standardize measurements between samples, a generalized otolith was used to define common reference points when measuring otolith dimensions. The terminology is that of Pannella (1980).

The typical sagitta of a juvenile sockeye is laterally compressed and elliptical viewed from the distal side (Fig. 1). The long axis is oriented with the rostrum anterior and postrostrum posterior. The convex proximal side is divided by the grooved sulcus acusticus. On the distal face the sulcal groove terminates anterodorsally as the excisura and posteriorly as the postcaudal trough. On the longitudinal axis of the otolith there are three major nuclei referred to as primordia (Neilson and Geen 1982). Additional minor nuclei (two to five) may be found anteriorly along the longitudinal axis.

The convergence of radial bands at the intersection of domes

of concentric growth usually provides cues to the position of the major nuclei and orientation of the vertical and horizontal axes. The anterodorsal band which intersects the excisura is particularly useful, indicating the position of the excisura on sagittae displaying no inflection or double inflections on the margin.

Various otolith measurements have been related to body size. Jonsson and Stenseth (1977) compared the relationship of length to three posterior radii of the sagitta otoliths of brown trout (*Salmo trutta*). Selecting the radii which gave the best correlation, six regression models were examined including a fourth-order polynomial equation. S. Campana (DFO, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.) has related the length of starry flounders (*Platichthys stellatus*) to three linear measurements of the otolith: total length, central nucleus to posterior edge, and peripheral nucleus to posterior edge. For chinook salmon (*O. tshawytscha*) juveniles, Neilson and Geen (1982) used the total length of the otolith minus the length of the rostrum. Wilson and Larkin (1982) measured a dorsal posterior radius at 40° from the longitudinal axis for sockeye fry, the axis defined as the line from the tip of the rostrum through the posterior nucleus.

The emergence of fry from the gravel of the spawning stream may occur after the deposition of a variable number of "daily rings" (Wilson and Larkin 1982) and is marked on the otolith by a narrow zone of discontinuity which may extend around the whole of the otolith or be exhibited in only portions of the periphery. It appears as a light zone, bordered by a distinct dark band (Fig. 2), and is referred to in this text as the emergence check. Marshall and Parker (1982) named it the "first-feeding check." In either case, it is usually distinguishable and is assumed to be coincident with emergence and the transition from yolk absorption to active foraging in the lake.

In the present study, five otolith dimensions were measured, the first three the radii emanating from the posterior major nucleus at 140, 160, and 200° from the longitudinal axis on which lie the three major nuclei (Fig. 1). The fourth dimension was total otolith length, the distance along the longitudinal axis from a point perpendicular to the rostral inflection to the intersection of the axis and the posterior edge (Neilson and Geen 1982). The intersection was used regardless of the presence of lobes extending posterior to the intersection, or whether the postcaudal trough was not centered on the axis. The fifth dimension was a crude estimate of the saggital sectional area based on measuring 16 radii at 22.5° intervals and summing the areas of the enclosed triangles. Greater precision could have been obtained by planimetry (Habib 1976) or by digitizing on a computer graphics tablet. For the limited number of samples involved in this study, the method used was considered adequate.

To define the position of the axes prior to measurement, a template was positioned under the projected image so that the intersection of horizontal and vertical axes passed through the central nucleus and the vertical axis aligned with the vertical axis of the otolith. To measure posterior radii, the template was positioned with its origin at the posterior nucleus. Total otolith length measurements were made positioning the vertical axis of the template through the rostral inflection and measuring from the template origin to the intersection of the longitudinal axis and the edge of the postrostrum. For otoliths displaying multiple or no rostral inflections, an equivalent point was estimated by the intersection of the dorsoanterior radial band and the otolith edge (Fig. 1).

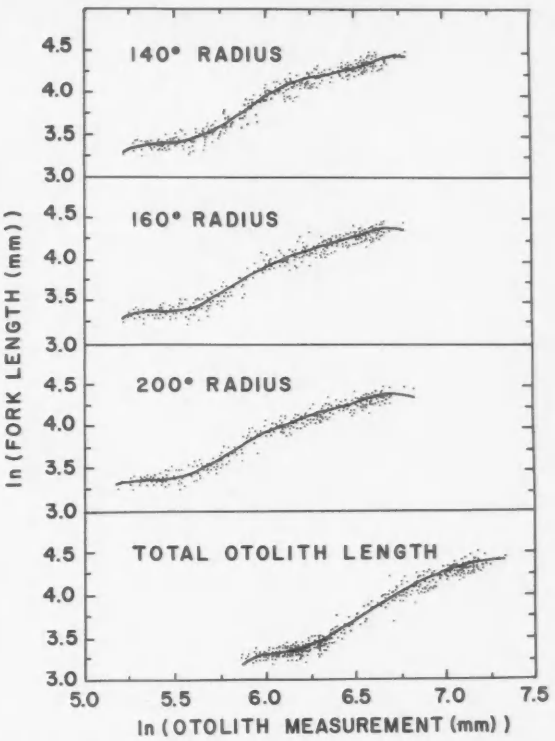

FIG. 3. Relationships between ln fork length and ln otolith measurement for 1978 brood juvenile sockeye from Babine Lake, B.C.

TABLE 1. Orthogonal polynomial coefficients for the relationships between ln fork length (mm) and ln otolith radius (μm) at 140, 160, and 200° and ln total otolith length (μm) for juvenile sockeye from the 1978 brood, Babine Lake, B.C.

Order of polynomial	Radius 140°	Radius 160°	Radius 200°	Total otolith length
Constant	−193382.89	−210289.34	−129298.33	−311595.70
1	193507.04	210537.62	130508.35	280521.51
2	−80506.644	−87647.458	−54758.770	−105070.85
3	17825.906	19421.103	12225.728	20958.907
4	−2215.6265	−2415.8643	−1531.9535	−2348.3730
5	146.57611	159.96699	102.15684	140.14400
6	−4.0324030	−4.4051223	−2.8324161	−3.4801885
R^2	0.9740	0.9726	0.9751	0.9823
S_{yx}	0.2496	0.2527	0.2466	0.2256

Dimensions of the enlarged images of the otoliths were measured to the nearest millimetre, giving an average precision of 0.7%. A second measurement error was associated with determining the exact position of the nuclei, but repeated tests using three independent measurements of the same otolith indicated that the smallest radii could be measured to within 1−2%, equivalent to about 2−3 μm.

Fork Length − Otolith Size Relationships

Over the 1-yr interval from fry to smolt, the form of the

TABLE 2. Predictive (P) and functional (F) regressions (Ricker 1973) of ln fork length (mm) on five otolith dimensions (μm) (cross-sectional area (A), total otolith length (T), and posterior radii (R)) at 140, 160, and 200° for a representative sample of emergent sockeye fry from the Fulton River and Pinkut Creek systems. All regressions are significant at $p \leq 0.01$.

Otolith dimension	Regression	r^2	n
Cross-sectional area	$\ln(L) = 0.3220 + 0.2514 \ln(A)$ (P)	0.5219	71
Cross-sectional area	$\ln(L) = -0.8510 + 0.3480 \ln(A)$ (F)		
Total length	$\ln(L) = 1.6447 + 0.2842 \ln(T)$ (P)	0.2501	71
Total length	$\ln(L) = -0.1001 + 0.5683 \ln(T)$ (F)		
Radius 140°	$\ln(L) = 2.3081 + 0.1992 \ln(R)$ (P)	0.1427	71
Radius 140°	$\ln(L) = 0.5269 + 0.5274 \ln(R)$ (F)		
Radius 160°	$\ln(L) = 2.4879 + 0.1659 \ln(R)$ (P)	0.1140	71
Radius 160°	$\ln(L) = 0.7194 + 0.4914 \ln(R)$ (F)		
Radius 200°	$\ln(L) = 2.3730 + 0.1878 \ln(R)$ (P)	0.1271	69
Radius 200°	$\ln(L) = 0.5406 + 0.5267 \ln(R)$ (F)		

TABLE 3. Predictive regressions for the relationship between ln fork length at emergence (x) and ln total otolith length (y) at five fry sources on the Babine Lake system. Slopes that are underlined are not significantly different from each other (Scheffés multiple range test, $p = 0.05$). The main lake regression was calculated from the combined fry sources, weighted by relative fry production.

Source	Pinkut Channel	Fulton River	Fulton Channel 2	Pinkut Creek	Fulton Channel 1	Main lake
Intercept	2.38017	1.86284	1.96408	1.26758	0.87015	1.52293
Slope (b)	0.15850	0.24940	0.23500	0.34607	0.40849	0.30493
S_{xx}	0.03523	0.03096	0.03152	0.03716	0.04537	0.03717
R^2	0.0856	0.2265	0.1572	0.2633	0.3577	0.2152
$t(b)$	3.18	6.67	6.39	7.86	9.35	14.97
$p(b = 0)$	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
n	110	154	221	175	159	819
y	3.34716	3.39146	3.40723	3.38095	3.37263	3.38786
S_y	0.03667	0.05643	0.04318	0.03509	0.03426	0.04603

various fork length versus otolith measurement relationships was moderately sinuous (Fig. 3). During the juvenile period the otolith grows at a more rapid rate than fork length, a positive allometry that would give way to a negative allometry at later stages in the life history (E. B. Brothers, 3 Sunset West, Ithaca, NY 14850, pers. comm.). The data transformed by natural logarithms to make variances homogeneous and fitted with stepwise orthogonal polynomials resulted in 8th- and 12th-order equations with some coefficients approaching magnitudes of 10^{14}. Although the regressions were highly significant with R^2 values ranging from 0.96 to 0.98, they were to some extent an artifact of the sample size (Draper and Smith 1966). Third-order polynomials were significant with R^2 values of approximately 0.94, but the residuals were not normally distributed. Sixth-order orthogonal polynomials were a useful compromise, being significant ($p < 0.01$) for all otolith measurements with R^2 values ranging from 0.97 to 0.98 (Table 1). Although the polynomial expressions were used for back-calculation over short intervals in the midrange of the fry to smolt period, there were substantial errors when the back-calculation extended to the ends of the range of observations.

For estimating fork length at emergence, it was found to be preferable to use the relationship between fork length and otolith size of emergent fry coupled with that for fish of the time period from which length at emergence was to be back-calculated. For back-calculation of fork length, the deviations from the predictive function at the time of capture were assumed to be constant over the back-calculated period and estimated as ln fork length at capture minus the predicted ln fork length at capture (Carlander 1981).

A subsample of 71 emergent fry from the Fulton River and Pinkut Creek systems indicated significant predictive and functional linear correlations (Ricker 1973) between fork lengths and the five otolith measurements (Table 2). Sagittal section area was the best estimator of fork length, perhaps reflecting that daily growth increments are not always distributed in the same way around the periphery of the sagittal plane, where their width is greatest (Dunkelberger et al. 1980), and that an integration of the increments along all radii is accordingly more representative of the daily growth of the otolith. For the three posterior radius regressions the correlations were poor ($r^2 = 0.11-0.14$). Total otolith length ($r^2 = 0.25$) was a better estimator of fork length. There is evidently considerable variability in fork length for a given otolith size. However, total otolith length is measured more easily and with greater precision than sagittal section area, and the emergence check is discernible at the excisura and postcaudal groove of most otoliths.

Stock differences in the body length – otolith dimension relationships at emergence were examined with samples com-

TABLE 4. Predictive linear regressions of ln fork length at capture (Fc) on ln total otolith length at capture (Tc) for 1978 brood sockeye in mid-July, mid-August, mid-September, and at the sub-2 smolt emigration from the main basin of Babine Lake.

Life history stage	Predictive regression	r^2
mid-July	ln (Fc) = −4.08384 + 1.19502 ln (Tc)	0.6422
mid-August	ln (Fc) = −2.43577 + 0.95689 ln (Tc)	0.6295
mid-September	ln (Fc) = −1.14453 + 0.76759 ln (Tc)	0.4510
Smolt	ln (Fc) = 0.77640 + 0.49969 ln (Tc)	0.3136

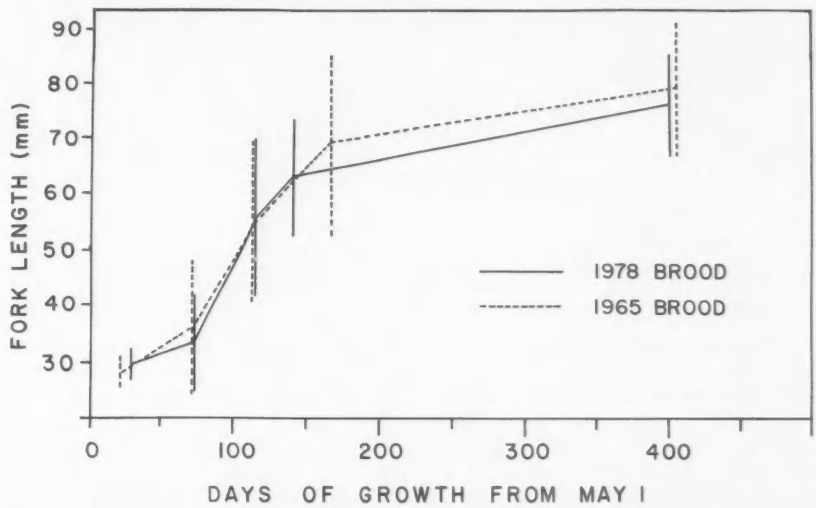

FIG. 4. Mean fork length of 1978 and 1965 brook sockeye (McDonald 1969) from fry emergence to smolt emigration from Babine Lake, B.C. Vertical bars represent two standard deviations on either side of the mean.

prising subsamples of approximately 25 fry from each source, taken three times weekly, over the period of fry migration. The regressions were significant for all stocks, but for Pinkut Channel and Fulton Channel 2, the relationship was highly variable (r^2 = 0.0856 and 0.1572, respectively). Scheffés multiple range test indicated two homogeneous subsets with respect to slope (Table 3). Within the larger subset there were three subsets with respect to adjusted ln mean fork length: the Fulton River and Pinkut Creek samples forming one subset, the other two, Pinkut Channel and Fulton Channel 2, each comprising separate subsets. Within the second subset the two subsamples, Pinkut Creek and Fulton Channel 1, were significantly different with respect to adjusted ln mean fork length. Thus, there is no obvious grouping of channel fry as opposed to river fry or of Fulton fry as opposed to Pinkut fry.

The combined regression for the main lake was obtained by weighting the samples from each source by the relative 1978 brood fry production. Fry production from the small streams which were not studied was assumed to be equal to 233 fry per spawner (McDonald and Hume 1984) and fork length − otolith relationships assumed to be most similar to those from Pinkut Creek (Table 3).

The combined average regression was highly significant ($p < 0.001$, Table 3). Residuals from the average regression were homogeneous and normally distributed. Use of the average regression gave minor loss of precision: the mean increase in standard deviation from regression, weighted by relative fry production, was less than 2% for each stock. In back-calculations, the average regression underestimated length at emergence in stocks for which the relationship was above the average regression and overestimated lengths for those below. The largest errors were associated with the Pinkut spawning channel stock, which has the relationship most different from the average regression. However, the bias (overestimated fork lengths) is small because the channel produced only 7.5% of the main lake fry population in 1979.

The combined average regression was used as the predictive function for back-calculation of ln total fork length at emergence. Regressions for each of the other life history stages (Table 4) were used as the functions for evaluating the deviation of the observed length at capture from the expected length at capture. This procedure assumes that deviations from the predictive relationships were constant over the range of ln total otolith length. That is, for fish of any given length, those with relatively large otoliths at emergence were assumed to have equally relatively large otoliths at subsequent stages in the life history. While this may not be true for individuals and might not be a valid procedure for back-calculating individual growth histories, it is satisfactory for describing the statistical characteristics of growth in the population.

To obtain for the main lake the distribution of total otolith length at emergence from the samples of emerging fry, the

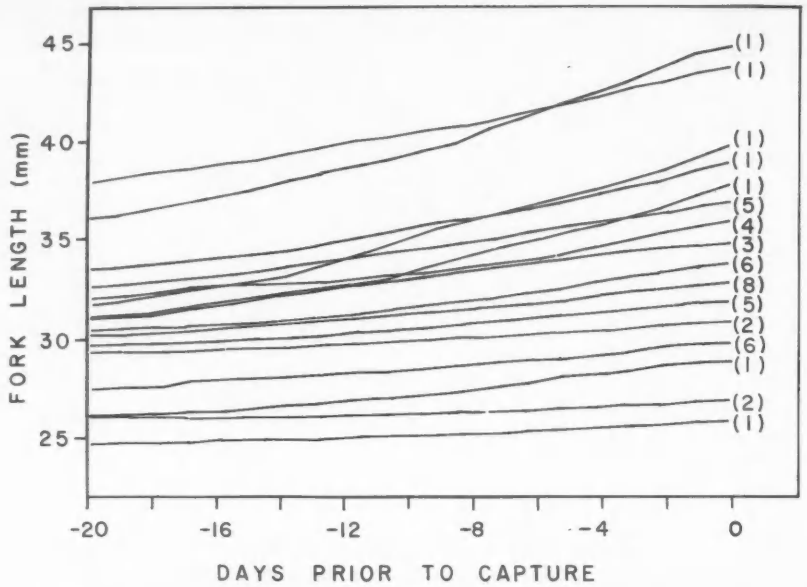

FIG. 5. For groups of individuals assembled at 1-mm intervals of fork length at capture, estimated mean fork lengths over the 20-d period prior to capture in July, the central capture date being July 12. Numbers in parentheses indicate sample size.

daily distribution of total otolith length was weighted by the daily count of fry for each source of fry. The daily distributions from all sources were then summed. A similar procedure was used to obtain the distribution of total otolith length at emergence from the daily samples of smolts at their single point of collection at the outlet of Babine Lake.

Results

Growth Rate in the Lake

The growth rate of the 1978 brood as indicated by the mean fork length at capture of main lake fry was similar to that for the 1965 brood (McDonald 1969) (Fig. 4). The range of fork lengths increased rapidly during the early lake-rearing period, indicating a large variation in growth rates of individual fry.

The relationship of growth rate to fish length in late June and early July was assessed by measuring the growth for the 20 d prior to capture in the otoliths of the July 12 tow samples and back-calculating fork lengths using the sixth-order polynomial for the relationship between fork length and the 140° radius measurement of the otolith. Mean growth increments over the 20-d period for groups of individuals at 1-mm intervals of fork length at capture show an orderly divergence (Fig. 5), implying a relationship between growth rate and fork length that would lead to maintenance of a hierarchy of lengths. The correlation of instantaneous daily growth rates and ln fork length was significant ($r^2 = 0.6142$).

Selective Mortality in the Lake

The distributions of total otolith length at emergence for the main lake fry, tow-netted juveniles, and smolts exhibit a trend for fish with small otoliths at emergence to disappear between the fry and smolt stages (Fig. 6). The mean total otolith length at emergence for emerging fry was 458.1 μm, and 47.5% were smaller than the mean. For the smolts, only 11.3% had otoliths less than 458.1 μm in total length at emergence. Fry to smolt survivals of those below and above the mean of total otolith length at emergence were 8.9 and 63.6%, respectively, and correspond to instantaneous mortality rates of 2.42 and 0.45. The resulting shift in the mean total otolith length at emergence from 458.1 to 493.6 μm was highly significant (ANOVA, $p < 0.001$). The shift was most pronounced in the latter part of the summer and the early autumn.

The relative frequencies of back-calculated fork lengths at emergence (Fig. 7) indicate selection against fry of small fork lengths. The increase in mean fork length at emergence from 29.55 to 30.40 mm (difference 0.85 mm, $s^2 = 1.352$) between the fry and smolt stages was statistically significant (ANOVA, $p < 0.001$), and most of the change occurred in the late summer and early autumn. Fry to smolt survivals of those above and below the mean of the distribution of mean fork length at emergence were 27.2 and 43.4%, respectively, corresponding to instantaneous mortality rates of 1.31 and 0.83.

The greater degree of selection against fish with relatively small otoliths than of fish of lesser fork length may reflect the lack of precision and perhaps a bias in the back-calculation procedure, but another possible explanation is that otolith length may be better correlated with weight than length of the fish. Thus, relatively short but heavy fish would have large otoliths and would have lower mortality rates than implied by their length. Further, the greatest mortality would be among the relatively shorter and lighter fish.

Discussion

It is well known that growth rate varies within populations

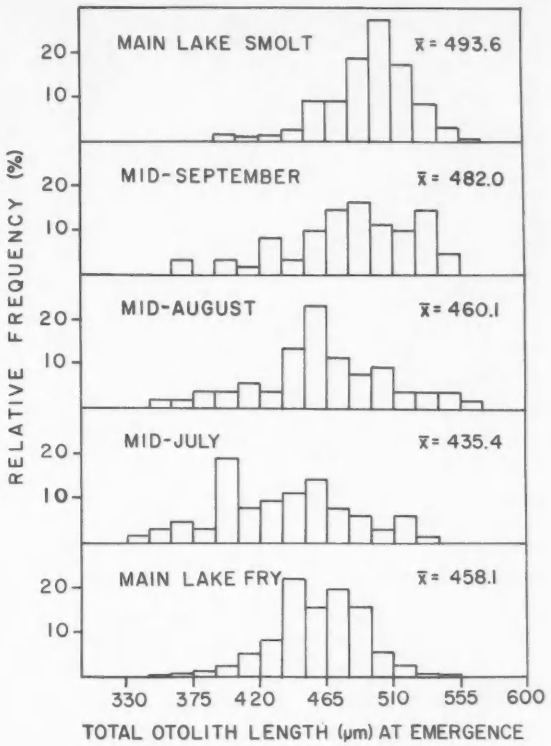

FIG. 6. Histograms of total otolith length at emergence for fry, for juveniles in mid-July, mid-August, and mid-September, and for sub-2 smolts for the 1978 brood sockeye from the main basin of Babine Lake, B.C.

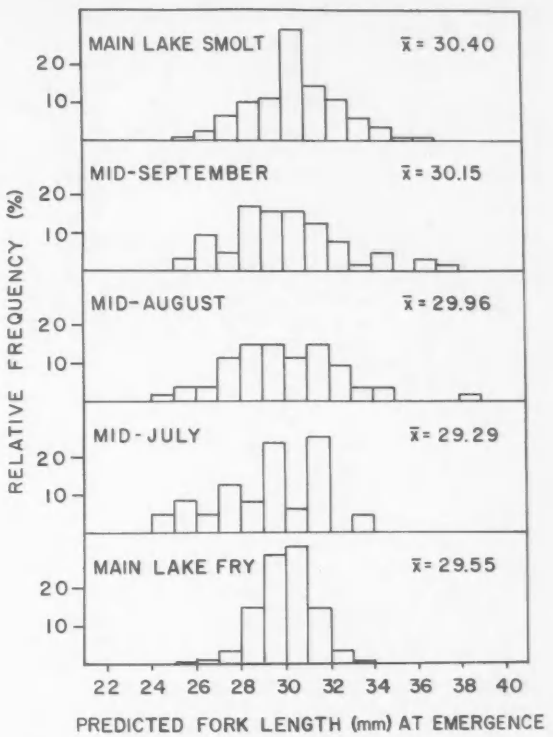

FIG. 7. Histograms of predicted fork length at emergence for fry, for juveniles in mid-July, mid-August, and mid-September, and for sub-2 smolts for the 1978 brood sockeye from the main basin of Babine Lake, B.C.

of juvenile salmon. Differences in egg size, emergence timing, together with spatial and temporal variation in environmental conditions, contribute to differences in size within cohorts. Competition may exaggerate variability in size and growth rate. DeAngelis and Coutant (1979) found that growth rate of juvenile smallmouth bass (*Micropterus dolomieu*) on surplus ration was independent of size, which was normally distributed. With competition for food, growth rates are more variable and size distributions may become bimodal (Shelton et al. 1979; Timmons and Shelton 1980; DeAngelis and Coutant 1982). Mason (1969) found that initial differences, compounded by competition through territoriality, were instrumental in determining social status, growth, and emigration rate in juvenile coho (*O. kisutch*).

Initial size differences of <1 mm fork length may thus have profound implications for growth and mortality of individuals within cohorts of juvenile sockeye. The linear dependence of growth rate on fork length of juvenile sockeye during July is not surprising and presumably reflects a competition for food resources. The disparity in estimated growth rates of Babine sockeye juveniles is considered to be conservative. Starved fish develop a body length − otolith relationship of lower slope than fed fish (Marshall and Parker 1982); hence, our use of an average slope underestimates the growth of faster growing fish and overestimates that of slower growing fish, biases which would reduce disparity among estimated growth rates. The relationships between body weight and otolith dimensions may be more precise for describing growth.

From observations on five broods of sockeye, McDonald (1969) and McDonald and Hume (1984) concluded that there was no size-selective mortality between pairs of stocks from the Fulton and Pinkut systems despite initial differences in mean size, which is seemingly contradictory to the present results. However, the mean may not necessarily reflect the history of individuals within the population.

Ricker (1969) showed that the change in instantaneous mortality rate resulting from linear change in selective pressure may be calculated as the ratio of the shift in mean length and its variance. The observed shift in mean length at emergence observed in this study was 0.85 mm with variance 1.352, implying a change in instantaneous mortality rate of 0.63/mm. Multiplying this estimate by the difference in fork length in any pair of observations enables an estimate of the survival rate of one of the pair from that of the other. Table 5 provides estimates of the increased survival rate for the five pairs reported by McDonald and Hume (1984). While it is inherent in the calculation that the change will always be in the right direction, there is nevertheless a fair measure of agreement between the observed and predicted survival rates, especially bearing in mind that the change in selection pressure may not be a linear function of the shift in mean length. Hence, while there may be significant selective mortality against small indi-

TABLE 5. Mean fry lengths (West 1978), fry to smolt survival rates, and survival rate of the larger predicted from the smaller of each pair using a change in instantaneous mortality rates of 0.63/mm.

Brood year	Group	Emergent length (mm)	Fry to smolt survival (%)	Predicted survival rate (%)
1965	Fulton River	29.3	12.7	
	Fulton Channel 1	29.1	14.3	16.4
1966	Fulton River	29.4	25.8	25.4
	Fulton Channel 1	29.0	19.9	
1967	Fulton River	29.3	29.3	
	Fulton Channel 1	29.8	33.2	40.2
1970	Fulton Channel 2	30.3	25.0	25.4
	Pinkut Channel	30.2	23.9	
1971	Fulton Channel 2	30.0	37.2	33.0
	Pinkut Channel	29.6	25.6	

viduals within a population, selection might not be obvious from comparison of two populations undergoing a common selection.

Parallel changes in rates of growth and survival are often postulated (for example, Isaacs 1964) but there is little direct evidence of their occurrence. Methot and Kramer (1979) examined the possible effect of selective mortality on estimates of growth rate of northern anchovy (*Engraulis mordax*) larvae based on relating standard length to the number of daily otolith increments, concluding that the effects were not sufficient to introduce a substantial bias. It was also suggested that back-calculation of growth histories of individuals from daily growth rings could be used to provide information on differential mortality. Healey (1982) demonstrated size-selective mortality in chum salmon (*O. keta*), and Parker (1971) suggested that early marine mortality of juvenile chum and pink salmon (*O. gorbuscha*) from predation by juvenile coho was biased toward smaller individuals. The present findings were unequivocal: there is a selective mortality of fish that were relatively small at the time of emergence as fry. Those that start small tend to stay in the lower end of the size distribution and become progressively smaller compared with larger individuals at the other end of the size distribution. Differential mortality is apparently most intense in the latter part of the summer and in early autumn.

The proximate causes of mortality are presumably size related, such as predation, or condition related, such as disease and parasitism. The likelihood of death literally from starvation appears unlikely. Sockeye juveniles reared in laboratory conditions until November withstood 20 wk of starvation without significant mortalities (Bilton and Robins 1971), and although the fish were larger than juveniles in Babine Lake and were being held at a later season, their durability was impressive.

Size-related mortality in Babine juvenile sockeye may be induced by parasitism of the cestode *Eubothrium salvelini*. The susceptibility of fry to infection by the parasite declines sharply as fork length of sockeye juveniles increases above the range of 35–45 mm (Boyce 1974). Larger and faster growing fry move quickly through this size range, and hence may be less prone to debilitating effects of the parasite. Although infected juveniles may survive to the smolt stage, in laboratory conditions they are less able to cope with stress and exhibit reduced growth, swimming performance, and survival (Boyce and Behrens Yamada 1977; Boyce 1979, 1982; Boyce and Clarke 1983). Smith (1973) suggested that such indirect effects on juvenile quality could cause mortality from other sources. Higher predation rates on the smaller and less vigorous parasitized fish in the lake are an additional possibility.

Acknowledgments

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Separation of Aquatic Lipid Classes by Chromarod Thin-Layer Chromatography with Measurement by Iatroscan Flame Ionization Detection

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Parrish, C. C. 1987. Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44: 722–731.

Lipid classes in solvent extracts of marine dissolved and particulate matter, benthic invertebrates, net-caught zooplankton, and freshwater sediment trap material were measured with the Chromarod–Iatroscan (thin-layer chromatography (TLC)/flame ionization detector (FID)) system. As many as 11 classes were identified in these samples by combining multiple development schemes with the partial scanning facility in the Iatroscan. Part of the Iatroscan's circuitry was modified to include a scan-interruptor switch to further facilitate partial scanning. Quantitative aspects of the TLC/FID system were improved with the use of a syringe fitted with a repeating dispenser for sample application, and by maximizing the detector's response. High FID responses were obtained with double developments and high hydrogen flow rates and FID scan rates. Under these conditions an analytical precision of 10% was attained for most compounds with only about five analyses on different Chromarods, and the range in mean FID response to different compounds was significantly narrowed. An intercalibration was performed to test TLC/FID accuracy: the Iatroscan gave lipid values which were about 85% of true gravimetric values.

À l'aide du système Chromarod-Iatroscan (chromatographie en couche mince (TLC)/détecteur d'ionisation de flammes (FID)), on a quantifié les lipides dans des extraits par solvants de matières marines dissoutes et particulaires, d'invertébrés benthiques, de zooplancton capturé au filet et de sédiments recueillis en eau douce. On a ainsi identifié jusqu'à 11 classes de lipides dans ces échantillons en combinant des systèmes de développement multiple avec la capacité de balayage partiel de l'Iatroscan. On a modifié une partie du circuit de l'Iatroscan pour inclure un interrupteur de balayage afin de faciliter le balayage partiel. On a aussi amélioré les aspects quantitatifs du système TLC/FID, avec l'utilisation d'une seringue équipée d'un distributeur à répétition pour le dosage des échantillons et la maximalisation de la réaction du détecteur. On a ainsi obtenu des réactions élévées du FID avec des développements doubles en présence de taux élevés du débit d'hydrogène et de balayage FID. Dans ces conditions, on a obtenu une précision analytique de 10 % pour la plupart des composés avec seulement cinq analyses environ sur différents Chromarods; l'écart de la réaction moyenne du FID en présence de différents composés a été nettement resserré. On a effectué un étalonnage intermédiaire afin de vérifier la précision du TLC/FID : dans le cas des lipides, l'Iatroscan a donné des valeurs qui se situaient à environ 85 % des vraies valeurs gravimétriques.

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The Chromarod–Iatroscan system is based on two well-established analytical tools: thin-layer chromatography (TLC) and the flame ionization detector (FID) used in gas chromatography (GC). The Chromarods are reusable silica gel coated rods (Ackman 1985) on which TLC separations are performed. The Iatroscan houses the FID and the machinery used to pass the Chromarods sequentially through its flame and under its collector electrode. This new analytical system, known as TLC/FID, is now gaining popularity in environmental research, especially in marine lipid class work (Kellogg and Patton 1983; Morris et al. 1983; Parrish and Ackman 1983a; Vetter et al. 1983; Delmas et al. 1984; Hakanson 1984; Harvey et al. 1984; Sasaki and Capuzzo 1984; Fraser et al. 1985; Volkman et al. 1986).

In the past 5 yr, it has become clear that TLC/FID cannot be considered as simply a combination of traditional plate-TLC and GC/FID techniques. It is an analytical system in its own right; there has been extensive research specifically into Chromarod-TLC separations (e.g. Kramer et al. 1980, 1985; Parrish and Ackman 1983a; Tatara et al. 1983; Murray 1985) and into Iatroscan-FID responses (e.g. Farnworth et al. 1982; Crane et al. 1983; Parrish and Ackman 1983b, 1985; Kaimal and Shantha 1984; Rao et al. 1985).

The present study is the culmination of a series of experiments designed to establish separation and calibration procedures for the determination of marine lipid classes in dissolved and suspended particulate matter (Parrish and Ackman 1983a, 1983b, 1985; Delmas et al. 1984). The methods described herein have now been used successfully in the analysis of over 500 different samples (Parrish 1986), and the types of samples have been extended to include freshwater samples, particulate matter from sediment traps, and benthic and pelagic invertebrate samples.

Gravimetry and GC have been the traditional mainstays of

quantitative work with lipid extracts. These techniques, however, do have their shortfalls. Gravimetric measurements can be easily biased upward (Hopkins et al. 1984), while total lipids obtained by GC are invariably considerably smaller than the actual lipid mass (Wakeham et al. 1980; Kennicutt and Jeffrey 1981a, 1981b). These techniques are also limited in their suitability for environmental and ecological interpretation. With measurements of total lipid by gravimetry, or fatty acid moieties by GC, it can be difficult to elucidate the nature and source of lipid material. By measuring lipid classes, it should be possible to distinguish energy storage classes, membrane classes, pollutants, and indicators of lipid degradation (Parrish 1986).

Methods

The analytical methodology leading to the measurement of aquatic lipid classes comprises several steps. These include sampling, extraction, storage, concentration, and analysis. Throughout these steps it is important to exclude contact with other lipid-containing materials, as well as to minimize exposure to light, oxygen, and heat: aquatic lipids are characterized by a high degree of polyunsaturation and can thus be quite labile. The methods summarized here are based on those of Delmas et al. (1984) and Parrish and Ackman (1985). They were described in detail by Parrish (1986).

Sample Workup

Seawater samples were screened through a 200-μm nylon mesh to remove large zooplankton and particles. They were then passed through precombusted (400°C) glass fibre filters; light suction was applied via tap water aspiration. The more commonly used Millipore pumping systems were used initially, but were avoided subsequently because of high hydrocarbon contamination.

Between 500 mL and 3 L of seawater was filtered for "particulate" lipid measurement, and between 200 mL and 1 L was filtered for "dissolved" lipid measurement. Wherever possible, filtrations and extractions were done in quadruplicate, and a total system blank was run for each set of extractions.

Dissolved lipids were extracted into dichloromethane (CH_2Cl_2). Particulate lipids were extracted into CH_2Cl_2–methanol (2:1). The methanol (MeOH) and the nonlipid material was back-extracted into "Super-Q" water (Millipore) which had been redistilled and passed through an oven at 800°C to further purify it. The two phases were separated by centrifugation. All subsequent extractions and transfers were done with 100% CH_2Cl_2 to minimize the amount of MeOH in the final concentrate.

Five to 20 μg of *n*-hexadecan-3-one was added to each sample before extraction. This internal standard was then extracted with the lipids in the sample, and the amount measured in the final concentrate provided an estimate of lipid recovery.

Benthic invertebrate samples and sediment trap samples were extracted into chloroform–MeOH (2:1) using a micromethod detailed by Gardner et al. (1985). About half of each sample was used for gravimetric determinations (Gardner et al. 1985) in order to provide a comparison between these measurements and total lipids determined with the Iatroscan.

A marine invertebrate sample was obtained by sonicating a 240-μm-mesh net-tow sample in CH_2Cl_2. The seawater–CH_2Cl_2–zooplankton mixture was filtered and transferred to a separatory funnel. Purified water was added and the sample was treated in a manner similar to that used for dissolved lipids.

Analytical Apparatus and Operating Conditions

Operating conditions for the Iatroscan MK III were adjusted to maximize FID response: a relatively high hydrogen flow rate (173 $mL \cdot min^{-1}$) and scan rate (4.2 $mm \cdot s^{-1}$) were used. For recording of chromatograms and for peak area integration, the Iatroscan was connected to a Hewlett-Packard 3390A computing integrator. The Iatroscan and the integrator were connected to a double-pole, double-throw scan-interruptor switch which could be used at any time during a scan to move the rod out of the flame and to stop data collection.

Standards and Calibration

A stock solution of an eight-component composite standard, HC, WE, KET, TG, FFA, ALC, ST, and PL (abbreviations explained in Table 1), was made up in chloroform ($CHCl_3$) and stored under nitrogen at −20°C. Several different dilutions of this standard were made and these were stored in 3-mL glass microreaction vessels (Supelco) fitted with Teflon Mininert valves (Supelco). Standards used daily ranged in concentration from 0.2 to 15 $\mu g \cdot \mu L^{-1}$. They were regularly renewed from dilutions of the stock solution. Before making the composite standards, each component was run singly through the entire development procedure to determine purity. Whenever a large set of samples was to be analyzed, calibration curves were compiled throughout the whole period of sample analyses. With each set of 10 rods, one rod, chosen randomly, was used for standards on each day of analyses. The load applied to the rod ranged from 0.4 to 20.0 μg.

Standard and Sample Application

Standards were routinely spotted onto the SII Chromarods using Hamilton syringes fitted into Hamilton repeating dispensers. When applying samples or standards to the rods, the frame holding the rods was placed on a warm hot-plate with the lower edge extending beyond the end of the hot-plate so that the point of application at the bottom of the rods was not directly over the heat source. This helped keep the solute in a narrow band near the end of the Chromarods. Solvent focusing (Harvey and Patton 1981; Parrish and Ackman 1983a, 1983b) was also employed for this purpose. Solvent focusing is useful when a large volume (>10 μL) needs to be spotted on any one Chromarod. Any solute that has spread away from the point of application is refocused into a narrow band by developing in a polar solvent to just above the point of application.

Chromarod Conditioning and Cleaning

After spotting and before each development, the rods were conditioned for 5 min at a constant humidity (~30%). At the end of each developing session, after the last complete scan of each rod, the Chromarods were removed from their frame and placed in chromic – sulphuric acid. They were left there for at least 1 h before being thoroughly washed with Super-Q distilled water and then acetone.

TABLE 1. Compounds representing aquatic lipid classes that can be resolved in a three-step separation. Representatives in the second compound column cannot be completely resolved from representatives in other classes using the solvent systems described in the text.

Class	Abbreviation	Principal representative compound	Other representative compound
Aliphatic hydrocarbon	HC	*n*-Nonadecane	Squalene
Polycyclic aromatic hydrocarbon	PAH	Phenanthrene	Anthracene
Wax ester/ sterol ester	WE	Octadecyl hexadecanoate	Cholesteryl hexadecanoate
Methyl ester	ME	Methyl docosanoate	Methyl dodecanoate
Ketone	KET	Hexadecan-3-one	Octadecan-3-one
Acylated glyceryl ether	GE	Glyceryl-1-octdecyl ether, 2,3-dioctadecanoate	
Triglyceride	TG	Glyceryl trihexadecanoate	Glyceryl tridodecanoate
Free fatty acid	FFA	Hexadecanoic acid	Dodecanoic acid
Free aliphatic alcohol	ALC	Hexadecan-1-ol	Phytol
Free sterol	ST	Cholesterol	Brassicasterol
Diglyceride	DG	Glyceryl-1,2-dihexadecanoate	Glyceryl-1,3-dihexadecanoate
Acetone-mobile polar lipids	AMPL	Glyceryl-1-monohexadecanoate	Chlorophyll *a*
Phospholipids	PL	Phosphatidylcholine	Sphingomyelin

Developments and Partial Scans

Four different solvent systems were used to obtain three chromatograms per rod. The first solvent system, hexane–diethyl ether–formic acid (99:1:0.05), was used for a double development: 25 min followed by 20 min. Each rod was then scanned to the lowest point behind the KET peak to obtain the first chromatogram. The second chromatogram was obtained after a 40-min development in hexane–diethyl ether–formic acid (80:20:0.1); this time the scan was continued to the lowest point behind the DG peak. The last chromatogram, a complete scan, was obtained after two double developments. The first was in 100% acetone (15 min for each development) and the second was in CH_2Cl_2–MeOH–water (5:4:1). The last developments were 10 min each.

Results and Discussion

The Chromarod–Iatroscan system consists of two independent parts. Up to 10 rods are grouped together in frames, and each frame is usually considered as a unit. One set can be scanned while developing another. This is useful because development times can be quite long. It is also possible to develop several sets simultaneously; thus, long developments are not a limitation in terms of sample processing. It is the extraction and concentration stage that is the slow step in the entire procedure leading to the determination of lipid classes. Every effort was thus made to simplify this stage and to make it as rapid as possible, while maintaining maximal extraction efficiencies and recoveries, together with minimal artefact formation.

Extraction and Storage of Seawater Lipids

$CHCl_3$ was used initially to extract the lipids from both dissolved matter and suspended particulate matter. $CHCl_3$ was later replaced by CH_2Cl_2 which has been suggested as an alternative to $CHCl_3$ in the extraction of lipids because it is less hazardous to human health (Chen et al. 1981). These workers found that CH_2Cl_2 was equally as effective as $CHCl_3$ in extracting various lipid classes from food products. Comparisons between $CHCl_3$ and CH_2Cl_2 for seawater extractions indicated that they were equally effective at extracting marine lipid classes. Since CH_2Cl_2 is more volatile, it also permits a faster concentration of solutions at a lower temperature than is feasible with $CHCl_3$. When it is necessary to spot large volumes (>10 μL) of solutions, CH_2Cl_2 is again preferable, since the solvent evaporates more rapidly from the silica surface on the Chromarod. Spotting is thus faster and the spreading of solute away from the origin is less.

All samples were stored in CH_2Cl_2, in dilute solution, under nitrogen, in the dark, and at −20 to −35°C. These conditions are very similar to those that have been recommended recently for the storage of marine invertebrate lipids (Sasaki and Capuzzo 1984). In the case of marine particulate lipids the entire filter was stored in this way until analysis. Immediately before analysis, the CH_2Cl_2 was removed and the filters were ground in CH_2Cl_2–MeOH. As much as possible of the MeOH

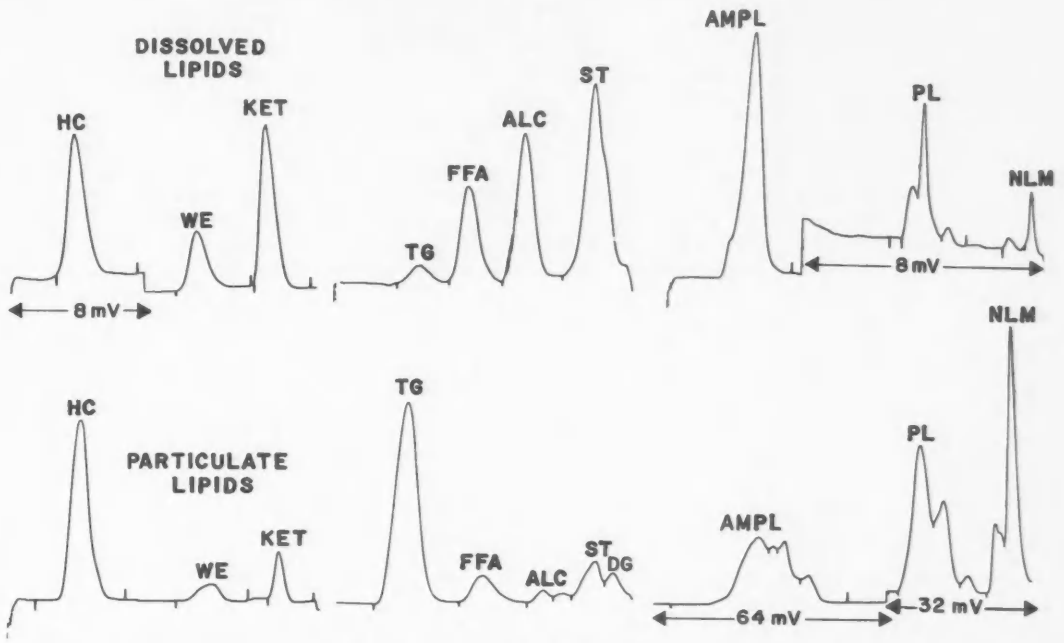

FIG. 1. Marine lipid class chromatograms from extracts of operationally defined (Whitehouse et al. 1984; Parrish 1986) dissolved and particulate matter. Each rod is scanned twice partially and once completely, resulting in three different chromatograms per sample application. The developing direction is from right to left; the scanning direction is from left to right. Lipid class abbreviations are explained in Table 1. NLM = nonlipid material; KET = spike added to each sample before extraction. Chromatograms were recorded at an attenuation of 16 mV full-scale deflection (FSD) unless otherwise indicated.

was removed with water and subsequent extractions were performed with CH_2Cl_2. All the CH_2Cl_2 extracts from one sample were combined and concentrated together.

CH_2Cl–MeOH has been used previously to extract lipids from phytoplankton (Volkman et al. 1980, 1981). In the case of marine particulate matter the inclusion of MeOH was found to be essential for maximizing the recovery of lipids from filters. This was especially true for phospholipids. Repetitions of the complete extraction procedure on previously extracted samples indicated that at least 80% of the recoverable lipids in each lipid class was obtained with the procedure described here.

Although MeOH is essential for extractions, it was an undesirable component in solvents coming into contact with samples after extraction. Samples stored in MeOH-containing solvents for long (>1 mo) periods showed a significant increase in ME content. In some marine samples, levels were high enough to interfere with the quantification of the internal standard peak (KET). Thus, efforts have been made to reduce the level of exposure of lipid extracts to MeOH after extraction. Esterification of carboxylic acid groups with MeOH has also been found to be a serious problem in extracts of airborne particulate matter (Clement et al. 1982). This artefact formation is also a widely acknowledged problem in other fields.

Further details related to sample extraction, including sample storage, cleaning of glassware, and blank levels, are provided in Parrish (1986).

Chromarod Separations and Partial Scanning

Figures 1 and 2 show the separations that may be obtained with partial scanning and the use of a sequence of solvent systems of increasing polarity. Eleven different lipid classes were identified in these aquatic samples, with 7–10 appearing together in any one sample. AMPL was one of the classes invariably present in aquatic samples; it was often the major class in seawater samples (Fig. 1). TG was another class that was always present; it was the major lipid class in *Pontoporeia hoyi* (Gardner et al. 1985) and in phytoplankton at the end of spring blooms (Parrish 1987). TG is an important energy storage class. ALC was a class that was not detected in all extracts (e.g. Fig. 2a, 2c) and was usually a minor component whenever present. WE was not detected in some extracts (e.g. Fig. 2c) but was a major component in others (Fig. 2a, 2b). Wax esters are important energy storage compounds in zooplankton. GE (Fig. 2b) is also a zooplankton indicator class; it was rarely detected in seawater samples.

The first development is the most critical step in the multistep procedure used to give the chromatograms in Fig. 1 and 2. The choice of solvent system is based on detailed studies of the

effect of varying the proportions of hexane, diethyl ether, or formic acid on neutral lipid separations (Kramer et al. 1980; Parrish and Ackman 1983a). It is possible to measure marine FFA in the first scan (Delmas et al. 1984; Parrish and Ackman 1985), since FFA runs ahead of TG in nonpolar solvent systems, but it was eventually decided that it was better to measure it routinely in the second scan to avoid problems with cutting the scan behind large tailing FFA peaks. There are also problems, however, with measuring FFA in the second scan. It is rarely possible to quantify either TG or FFA accurately when both are present at loads higher than 10 μg. It is partly for this reason that a double development is not performed before the second scan. While double developments improve the separation of compounds near the point of application, the more mobile compounds near the end of the rod tend to be closer together than they would be with a long single development. A brief investigation into the use of isopropanol to increase the separation of TG from other compounds (Sasaki and Capuzzo 1984) indicated that the improvements would be negligibly small for hexane-based solvents. Thus, the only recourse found for situations where there were high levels of TG and FFA was to spot a smaller amount of the same sample in a second analysis.

Figure 3 is a single-step separation of the same sample measured in a multistep separation in Fig. 2b. It can be seen that considerably more information is available from the multistep separation and that as a result of this, it is possible to assign identities to peaks with greater confidence. More information is also available about the wax ester component itself. WE peaks from the net-tow sample and from sediment trap samples were often split (e.g. Fig. 2a) sometimes so that two peaks were clearly distinguishable (Fig. 2b). This could indicate the presence of short-chain esters in addition to WE. However, there is probably not much ME present since the R_f values (distances moved along the rod) suggest that the second component is slightly less polar than ME. Another possibility is a wax ester/sterol ester split but this is also thought to be unlikely, since it was not possible to achieve such a separation with standards (Table 1). It is probable that the splitting represents differences in unsaturation and/or chain length within the class: both these factors affect R_f (Kramer et al. 1985). Split TG peaks have been found in marine dissolved and particulate matter (Delmas et al. 1984; Parrish and Ackman 1985). This peak splitting within classes, together with slightly different R_f values depending on sample load and sample type, means that it is advisable to co-spot low loads of new sample types with authentic standards to confirm peak designations.

Although there is less information available with the simple approach used in Fig. 3, it is a good starting point for samples of unknown lipid content. With one-step separations, it is often possible to reduce the developing time without seriously affecting the separation. Thus, with a single frame of 10 Chromarods, it would be possible to quantify the major lipid classes in 10 different samples within half an hour of spotting.

A separation such as the one in Fig. 3 would also be adequate for quantifying wax ester content. WE is by far the major component in the sample (note attenuations in Fig. 2b), so that given the precision of FID responses (Table 2), the presence of other minor components under the same peak would not seriously affect quantification. However, for all sample types it is advisable to perform at least one extended analysis to gain greater confidence in peak identities.

Figures 1 and 2 also provide good examples of separations that could not be replaced by a single-step development. In TLC separations of neutral lipids, phospholipids are often left at the origin (Fig. 3). An FID scan of this material leads to an estimate of the amount of "polar lipid" in the sample. This material is given the same abbreviation as that used for phospholipids, and it is often tacitly implied that this PL measurement is directly representative of phospholipid content. However, the material remaining at the origin can often be subdivided into two or three major components (Fig. 1 and 2).

A multistep scheme has been used here to obtain a relatively pure band of phospholipids. It involves the use of acetone and a MeOH-containing solvent system and is based on procedures used in silicic acid column chromatography (Rouser et al. 1967; Christie 1973). Acetone separates glycolipids, pigments, and any remaining neutral lipids from phospholipids, and the polar variation of a phospholipid-developing solvent system used here moves phospholipids away from the point of application. The material left at the origin has been termed nonlipid material and is presumed to consist of traces of proteins, carbohydrates, and humic material.

Acetone has been used previously in marine lipid class separations on Chromarods (Delmas et al. 1984; Harvey et al. 1984), but for shorter developments. The time used here for the final developments is probably excessive for many samples (e.g. Fig. 2) and could be replaced by a single 10-min development followed by an 8-min development in the MeOH-containing solvent system. However, the particulate lipid sample in Fig. 1 shows a situation where the extensive development times are necessary. In order that the minor classes could be quantified in this scan, a high load was applied to the Chromarod and thus broad AMPL and PL peaks are present in the final scan. In addition, both these classes show some splitting into their component parts, but the developments were extensive enough to permit the identification of the three major classes and to allow a summing of the various parts of these classes for their quantification.

The multistep approach to obtaining a pure phospholipid value is not foolproof. As with any peak, an absolute positive identification of the various compounds within a PL peak is impossible, but an indication of the presence of nonphospholipid compounds has been observed in some particulate samples. With high sample loadings, some blue colouration has been seen in the PL band. This could indicate the presence of a blue rearrangement product of fucoxanthin (Jensen 1964; Hallegraeff 1981).

Multiple development schemes (Fig. 1 and 2) require extensive use of the stop-scan facility in the Iatroscan. To stop the scan at the right position, one of the rods which has been spotted with the sample must be completely burnt, and measurements have to be made on the chromatogram of the distance to the desired scan-stop point. After converting this distance to a distance to be burnt on the Chromarods, the Iatroscan's scan-stop screw can be set at the desired position for the remaining rods containing the same sample. This procedure has to be repeated whenever it is necessary to change the scan-stop position for other separations on other rods.

When using the manufacturer's stop-scan facility, a relatively large separation between classes is required to avoid carryover into the next development. This is because different rods often show slight differences in the R_f values and lengths of bands. This can result from using rods of different ages, or spotting different volumes of solvent or different loads of

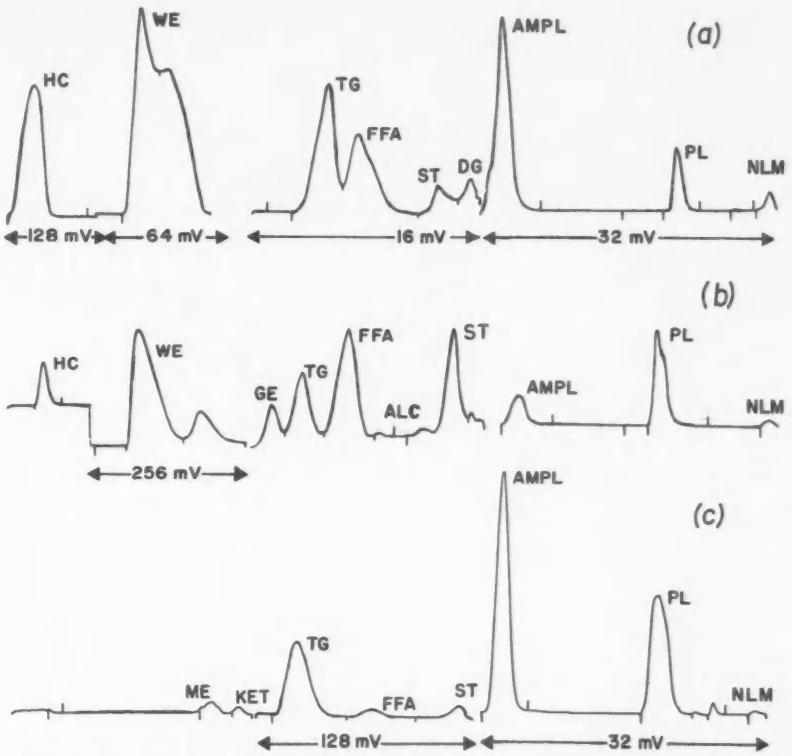

FIG. 2. Lipid class chromatograms from sediment trap and invertebrate samples. The developments and scans are the same as in Fig. 1. Lipid class abbreviations are explained in Table 1. NLM = nonlipid material; no internal standard was added to any of these samples. Chromatograms were recorded at 8 mV FSD unless otherwise indicated under the chromatogram. (a) Sediment trap sample from Lake Huron; (b) CH_2Cl_2 extract of zooplankton from a 240-μm-mesh net-tow near the edge of the Scotian Shelf; (c) $CHCl_3$–MeOH extract of *Pontoporeia hoyi* from Lake Michigan.

solute. For example, one could not halt the scan with the stop-scan facility between FFA and ALC in Fig. 1 and obtain complete scans of TG and FFA, and only TG and FFA, on more than a few rods. A simple way of overcoming these problems is to connect a switch to the Iatroscan's microswitch for stopping scans. Using this switch, a scan may be stopped at any position on any rod; the decision when to stop can be made according to the trace that is being produced on the chart-recorder during a scan.

Quantitative Determination of Aquatic Lipid Classes

The standards used to represent aquatic lipid classes (Table 1) were chosen on the basis of a literature review (Parrish 1986). In the belief that each rod should be considered as an isolated analytical unit, calibration curves were initially constructed for each standard on each individual rod of a set (Delmas et al. 1984). Subsequently it was found that the increase in precision over the use of interrod data (Parrish and Ackman 1985) was not enough to warrant the considerable extra effort involved in calibrating each rod individually. Thus, calibration data (Fig. 4) and estimations of analytical precision (Table 2) are obtained from all 10 rods within a set. However, before starting any extended period of TLC/FID analyses, rods with similar properties should be grouped together. By measuring a single compound on each rod of several sets after short developments in a polar solvent, an estimation of the magnitude of the response and the coefficient of variation (CV) can be quickly obtained for each Chromarod. The rods can be rearranged into sets of 10 of similar properties and each frame can then be considered as an analytical unit.

The precision of repeated analyses of standards used to be between 10 and 15% with about seven analyses per compound (Delmas et al. 1984; Parrish and Ackman 1985). These early CVs were obtained from Iatroscans operated at a lower scan rate and a lower hydrogen flow than was used in the present study, and all the standards were spotted with disposable capillary pipettes. The lower Iatroscan settings were used earlier in the belief that incomplete pyrolysis would be a problem at a higher scan rate (Crane et al. 1983) and that rod life would be reduced at a higher hydrogen flow (Ackman 1981). It now appears that these precautions were unnecessary. In the present study with high settings, it was possible to analyse over 500 samples on the one set of 10 rods and, except in the case of NLM, small peaks were detected in reburns only when high loads (>25 μg) of certain classes were pyrolyzed.

The higher settings used here were an attempt to increase FID response and to improve reproducibility (Ackman 1981). Double developments were performed for the same reason:

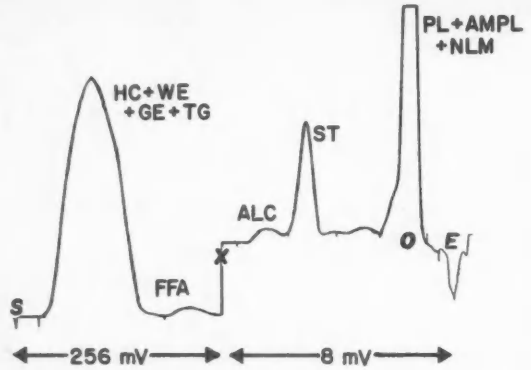

FIG. 3. One-step development and scan of marine zooplankton lipid classes (Fig. 2b). Peak identifications are given in Table 1. S and E denote the start and end of the scan; O is the point of application. Recorder attenuation was changed at X. The rod was developed from right to left for 40 min in hexane – diethyl ether – formic acid (80:20:0.1).

TABLE 2. Average precision of integrator areas obtained from scanning 0.4–20.0 μg of lipid compounds.

Compound	Average coefficient of variation (%)	Average number of analyses at each level
HC	7	4
KET	8	5
TG	8	4
FFA	16	4
ALC	8	3
PL	9	3
Pooled low-response calibration data	8	9
WE	11	4
SE	9	2
ST	8	3
Pooled high-response calibration data	11	6

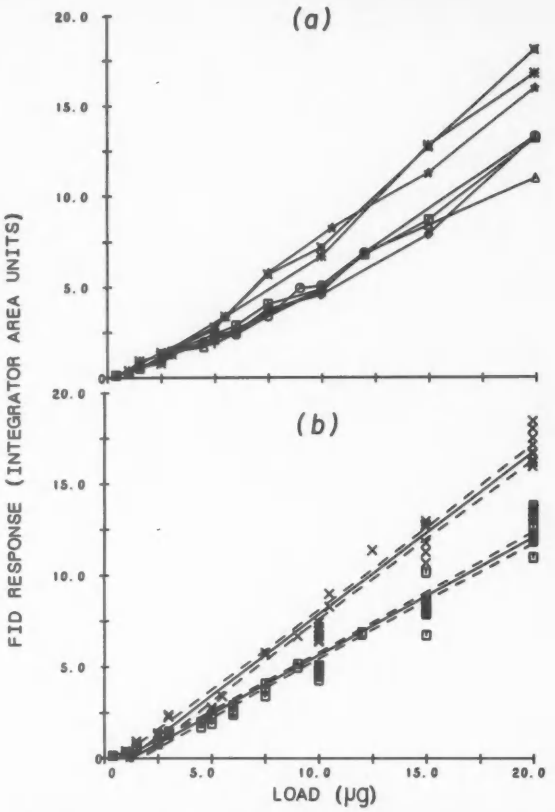

FIG. 4. Iatroscan calibration data for nine lipid classes. Compounds used to represent each class are given in Table 1. Additional developments and scans were performed to obtain calibration curves for steryl ester (SE). (a) Average responses from nine compounds. Each symbol represents a different lipid class. Levels at which average response data were available have been joined by straight lines. Above 10 μg, average response data are available for only seven classes; it was not possible to obtain a good separation between TG and FFA when each was present at loads above 10 μg. (b) Raw data from nine different compounds split into two groups. Crosses represent classes with high responses: WE, SE, ST; boxes are low-response classes: HC, KET, TG, FFA, ALC, PL. Linear regression lines are drawn through each group; broken lines are 95% confidence intervals for the regression lines.

FID response can be increased by producing narrower bands on Chromarods (Parrish and Ackman 1983b). The use of a second development in the same solvent system reverses the normal chromatographic process of band-broadening and thus a higher response is obtained. A double development was not performed before the second scan because it hindered TG/FFA separations at high loads and because precision was not improved in a previous study where a double development was performed before this scan (Parrish and Ackman 1985).

Table 2 suggests that there is an improvement in precision for individual compounds. The CV is now usually better than 10% with only about four analyses per compound. At least part of the improvement observed with most compounds is undoubtedly due to the use of a syringe fitted with a repeating dispenser for sample application, rather than the disposable capillary tubes that have been used in the past (Ackman 1981). A similar syringe application method reduced the standard deviation of Iatroscan measurements to as little as a third of the values obtained from the traditional method (Read 1985). The poor precision obtained with FFA (Table 2) appears anomalous; it may be related to the tendency of this peak to tail.

By comparison with the instrument precision given in Table 2, the total analytical precision for quadruplicate samples is usually between 15 and 30%. CVs even a high as 35% are not uncommon, especially for classes present in lesser amounts in extracts, or when less than four separate extractions were possible. This degree of imprecision might seem surprising at first, but it should be realized that even with the most entrenched or the most expensive equipment available, 20% total analytical precision is very common (e.g. Jungclaus et al. 1978; Goutx and Saliot 1980; Wakeham et al. 1980; Barrick 1982). Instrument precision is clearly not the weak step in the measurement of aquatic lipid classes. Problems with replication probably arise at the extraction stage, during storage, or

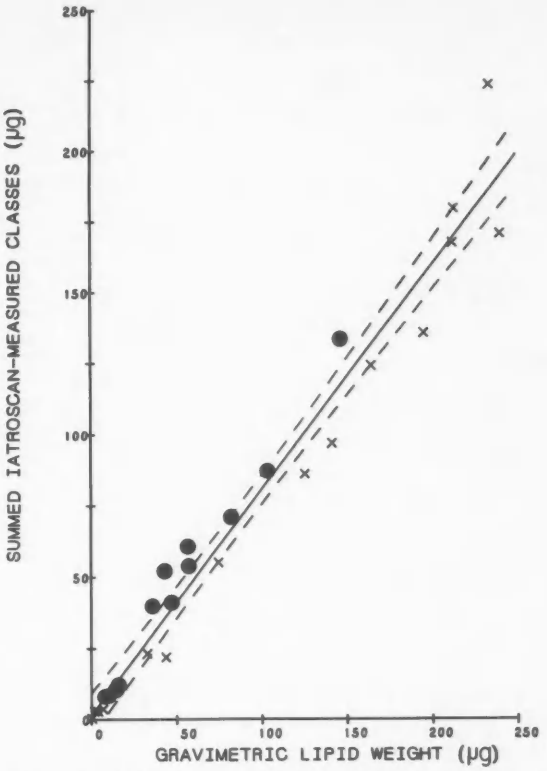

FIG. 5. Comparison of gravimetrically determined lipid weights with the sum of Iatroscan-measured classes. Solid circles represent Great Lakes benthic invertebrate samples; crosses represent Great Lakes sediment trap samples. The slope of the linear regression line is 0.8; broken lines are 95% confidence intervals for the regression line.

simply as a result of slight differences in sample handling.

Another reason for maximizing FID responses was that it was hoped that truly linear FID response curves could be obtained. A truly linear detector should obey a power law equation where the response index is between 0.98 and 1.02 (Scott 1983). If a plot of response versus load curves upwards as in Fig. 4, then the response index is significantly greater than 1.0 and the detector response is referred to as being "superlinear." There are two important consequences of the superlinearity of the Iatroscan-FID detector. The first is that multilevel calibration curves are essential and the second is that it is possible to get different responses to the same amount of compound depending on the width of the band on the Chromarod (Parrish and Ackman 1983b, 1985; Rao et al. 1985).

Despite all efforts, the calibration curves are still distinctly curvilinear (Fig. 4). Although the coefficients of determination (r^2) in Fig. 4b are both higher than 0.97, the lines clearly pass above the majority of the data points near the centre of the range and below the majority at both ends. As a result, these regressions could not be used for quantifying samples with class loads below 2.5 µg. It is for this reason that quadratic regressions are used for calibrations (Delmas et al. 1984; Parrish and Ackman 1985).

An unexpected benefit of the attempts to improve FID response is the close grouping of calibration data in Fig. 4. Previous calibration data were spread over a much broader range at each level (Delmas et al. 1984; Parrish and Ackman 1985). Beyond about 5 µg the data tend to fall into one of two groups (Fig. 4), so that it is possible to distinguish higher-response compounds (WE, SE, ST) and lower-response compounds (HC, KET, TG, FFA, ALC, PL). Within each group, calibration lines tend to cross and recross (Fig. 4a), suggesting that one calibration curve might suffice for low-response compounds and another might suffice for high-response compounds. This is supported by comparing the single-compound precision with the precision of all the data pooled together in the two categories (Table 2). The error bars for the pooled data are not usually any wider than for data obtained from a single compound. It is not known why WE, SE, and ST give a significantly higher response than the rest, but it should be noted that the proximity of the responses of these compounds to one another is less than for the low-responding compounds: the mean precision is not improved by pooling the data despite the larger number of analyses at each level (Table 2).

Kaimal and Shantha (1984) also found that several of the classes scanned on $CuSO_4$-impregnated Chromarods gave similar responses and had calibration curves which crossed one another. It would seem that the similarity in response to several compounds could be a fundamental property of Iatroscan measurements if efforts are made to maximize FID response. This could prove to be a valuable asset in the case of lipid classes where there may be a mixture of compound types (e.g. WE) or where the identity of the majority of the compounds is uncertain (e.g. AMPL). In both these classes there may be a significant level of a compound that has a structure different from that of the main contributor to the band on the Chromarod (Table 1). If the responses to different compounds within a single band on a Chromarod were very different, then it would not be possible to quantify these two particular chromatographic peaks in natural materials. This situation would be analogous to one that occurs in high-performance liquid chromatography (HPLC). The use of short-wavelength UV detection for quantification of HPLC-separated lipid classes is not possible unless there is a known or constant number of double bonds in each sample (Aitzetmuller 1982).

In TLC/FID studies involving aquatic lipid classes (Parrish 1986), WE and AMPL have been calibrated on the assumption that the components gave a high response. Stearyl palmitate and cholesteryl palmitate, which cannot be separated (Table 1), do indeed give similar high responses (Fig. 4), and chlorophyll *a*, which accounts for about 50% of the AMPL peak in *Phaeodactylum tricornutum* (Parrish and Wangersky 1987), has a response similar to ST (Delmas et al. 1984; Volkman et al. 1986).

The calibration data in Fig. 4 are different from previously published data not only because of the narrow range of responses but also because the relative responses of PL and WE have changed. Undeveloped PL peaks give a high response (Delmas et al. 1984), but after development the response is lower (Fig. 4), as would be expected from developed Chromarod bands (Parrish and Ackman 1983b). WE was subjected to a double development and so gives a higher response than when scanned after only a single development. PL was also developed twice, but it still has a lower response than undeveloped PL peaks; in terms of maximizing FID response, double developments are clearly not as effective

as no developments at all. However, precision is improved after developing because the material is more evenly spaced around the rod.

The range of responses in Fig. 4 corresponds to compounds of widely varying structure and molecular weight. This range indicates the bounds on the possible error in the estimation of lipid class content in a sample. If the range represents responses from the major nonvolatile lipids that could occur in an aquatic sample (Parrish 1986), then the possible error through uncertainty in the actual composition of a class should be relatively small. There is, however, one major difference between the standards used (Table 1) and aquatic lipids in general. Aquatic lipids, and especially marine lipids, have a relatively high proportion of polyunsaturated fatty acids, but because of lability, none is present in Table 1. However, Fraser et al. (1985) demonstrated that this should have little effect on TLC/FID calibration.

Figure 5 suggests that the range of responses in Fig. 4 is indeed a reasonable representation of responses to lipids in aquatic samples. There is a strong linear relationship (r^2 = 0.96) between gravimetrically determined lipid weights and Iatroscan measurements, and the intercept is not significantly different from zero. On average, the Iatroscan gives 84 (±9)% of the gravimetric value (n = 27). There is, however, clearly a dependence on sample type (Fig. 5), suggesting that the quantification procedures used here are, in the end, more applicable to some aquatic samples than to others. Nonetheless, the overall comparison is favourable, and it supports the value of 88.2(±2.0)% reported by Sasaki and Capuzzo (1984) for a similar intercomparison performed on a much larger number of marine invertebrate samples. The accuracy of TLC/FID appears to be equally as good on an individual class basis (Fraser et al. 1985). A value of 93(±16)% can be calculated from the data of Fraser et al. (1985) for TLC/FID versus combined gravimetric, colourimetric, and densitometric procedures for lipid classes. It is interesting that on average, TLC/FID seems to give slightly lower values than are obtained with other techniques. This may reflect the fact that TLC/FID is strictly applicable only to nonvolatile compounds: microgram loads of naphthalene and methylnaphthalenes give no response. These relatively volatile compounds presumably evaporate from the rods before the band is directly over the flame and under the collector electrode.

The accurate determination of lipid classes in aquatic samples does not depend entirely on an accurate calibration of the FID. It is also important to be able to determine the proportion of the sample lipids that are actually measured in a Chromarod scan.

For dissolved and particulate marine samples (Fig. 1), the use of an internal standard, *n*-hexadecan-3-one, has been found to be very useful for calculating lipid class concentrations in seawater. The term "internal standard" is a generic one: it has been applied to ketones added to seawater samples before extraction in order to measure recovery (Paradis and Ackman 1977; Delmas et al. 1984), as well as to ketones added to Chromarods to compensate for variations in detector response (Parrish and Ackman 1985). Keith et al. (1983) recommend the use of the term "surrogate spiking" for the addition of compounds in order to determine recovery efficiency.

An aliphatic ketone should probably not be used as a surrogate spike in samples containing a significant proportion of invertebrate lipids (Fig. 2). Large WE peaks (Fig. 2a, 2b) would cause problems with the quantification of KET, and there is a significant KET peak in the freshwater invertebrate chromatogram shown in Fig. 2c. TLC/FID analyses by Volkman et al. (1986) also indicated that KET could not be used for certain coccolithophorids, nor for anoxic surface sediments.

When only small amounts of sample are available, an alternative approach to using a surrogate spike is to use microtechniques requiring only small volumes of solvents (Gardner et al. 1985). The chromatograms in Fig. 2a and 2c were obtained from samples handled in this manner. This approach also permitted a direct comparison between gravimetry and Iatroscan measurements on these samples (Fig. 5).

Conclusion

Since the separation part and the detection part of the TLC/FID system are independent, and since it takes only 30 s to completely scan each rod, a large number of samples can be processed in a day. Several people can be working with different sets of rods and they may only require one Iatroscan for detection and quantification. In addition, experienced operators can work with two or three sets of rods at a time. This means that with even the most complex samples and the most involved separation and scanning procedures, the processing of 10–20 different sample extracts per person per day is a reasonable proposition. This is more than can be processed by GC or conventional HPLC. The initial outlay and the running costs are less than the GC or HPLC, and TLC/FID has the potential to measure a much broader range of compounds with considerable accuracy.

A major drawback of TLC/FID is that the detector is nonlinear so that calibration is more time-consuming and more complicated than in GC or HPLC. The detector is also orders of magnitude less sensitive than most detectors available for GC or HPLC, and TLC/FID analyses are less precise. However, with the advent of new detector designs and new sample applicators (Patterson 1985; Read 1985), this may soon change.

Acknowledgements

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Use of Echosounder Tracings to Estimate the Aboveground Biomass of Submerged Plants in Lakes[1]

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Duarte, C. M. 1987. Use of echosounder tracings to estimate the aboveground biomass of submerged plants in lakes. Can. J. Fish. Aquat. Sci. 44: 732–735.

A method is presented to estimate the aboveground biomass of submerged macrophytes in lakes from echosounder tracings and from the growth form of the dominant species in the stand. The equation is

$$\text{Biomass} = -343 + 37.7\,\text{height}^3 + 953(\text{form}\cdot\text{height})$$

where species that reach the surface to flower are categorized as form class 1, short understory species with floating flowers as form class 2, and species with underwater flowers, those lacking flowers, and those that, although able to produce flower, never flower in nature as form class 3. The standard error of the estimates is 421 g fresh wt.$\cdot m^{-2}$, a value comparable with the standard error of direct harvest by SCUBA divers. The echosounder-based method is limited to stands growing at depths greater than 70 cm with plants taller than 20 cm.

On présente une méthode d'estimation de la biomasse visible de macrophytes lacustres immergés à partir de relevés d'échosondage et de la forme de croissance de l'espèce dominante dans un peuplement. L'équation est la suivante :

$$\text{Biomasse} = -343 + 37{,}7\,\text{hauteur}^3 + 953(\text{forme}\cdot\text{hauteur})$$

où les espèces qui fleurissent à la surface sont classées dans la catégorie de forme 1, les espèces courtes de sous-étage avec des fleurs flottantes appartiennent à la catégorie 2, tandis que les espèces à fleurs immergées, celles sans fleur et celles qui, quoique capables de produire des fleurs, ne fleurissent jamais en milieu naturel sont classées dans la catégorie 3. L'erreur type des estimations s'éléve à 421 g poids humide$\cdot m^{-2}$, soit une valeur comparable à l'erreur type de la cueillette directe par des plongeurs en scaphandre autonome. La méthode basée sur les relevés d'échosondage est limitée aux peuplements situés à des profondeurs supérieures à 70 cm où la hauteur des plantes dépasse 20 cm.

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Submerged aquatic macrophytes, together with the phytoplankton, are principal contributors of autochthonous organic carbon to lakes. Because of their importance in the flux of energy (Adams and Prentki 1982) and their interactions with other biotic components, such as fish (Wiley et al. 1984), invertebrates (Wohlschlag 1950), and algae (Goulder 1969), the quantification of macrophyte abundance in lakes is important for limnology and lake management. However, in contrast with the phytoplankton, this quantification is not often done because not only are macrophyte distributions patchy within a lake, but their biomass within weedbeds is also highly variable (Downing and Anderson 1985).

A number of sampling techniques have been proposed to cope with the problem of quantitatively sampling the submerged macrophytes. Among the direct methods, quadrat harvests by SCUBA divers appear to be the most efficient (Downing and Anderson 1985) whereas mechanical samplers operated from the surface tend to overestimate biomass (Forsberg 1959). Among the indirect methods, those based on echosounder tracings of submerged beds or stands appear to be able to yield reasonable estimates of biomass (Maceina and Shireman 1980; Maceina et al. 1984). These workers estimated the mean biomass of submerged vegetation for entire lakes from echosounder tracings after calibrating these against a single biomass determination per tracing. Consequently, uncertainties were introduced into their model by errors in the single direct estimate of biomass and the single estimate of stand characteristics from echosounder tracings per site used to calibrate the method. While these uncertainties may be partially responsible for the large error of their biomass estimates, the authors point to changes in the biomass per unit height (B/H, grams per cubic metre) among stands as a major source of variance.

Since B/H of different species reflects their growth form (Lonsdale and Watkinson 1983), I tested whether the consideration of different form classes that reflect differences in B/H (C. M. Duarte and J. Kalff, submitted) will significantly

[1]Contribution 200 to the Lake Memphremagog project, McGill Limnology Research Centre.

TABLE 1. Mean and range for the SCUBA-obtained biomass (g fresh wt.·m^{-2}) and echosounder-derived height (cm) of the stands in the Eastern Townships (Québec) and in Lake George (New York) and the mean of the coefficients of variation (C.V.) for single estimates of the two variables.

	SCUBA biomass	Echosounder height
Mean	1335.6	122
Minimum	100.1	4
Maximum	5227.0	351
Mean C.V.	37.9	7.25

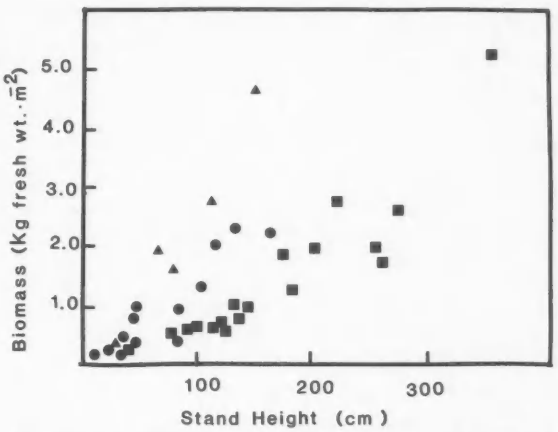

FIG. 1. Relationship between stand height and biomass for stands of nonflowering species (largely *Elodea canadensis*, ▲), short float flowering species (●), and tall plants with flowers at the surface (■).

improve the accuracy of echosounder estimates of submerged plant biomass over methods based on plant height alone. To do this, I compared the performance of biomass estimates obtained using both plant growth form and echosounder estimates of plant height with estimates obtained by direct SCUBA harvest. The comparison also permits an examination of the magnitude and properties of the errors involved.

Methods

The biomass of submerged vegetation was determined at 36 sites in nine different Québec lakes (45°N, 72°W) and in Lake George (New York, 43°N, 73°W) during late July and August 1985. The sites were selected to provide a wide range of depth, substrate, littoral slope, and exposure and also to cover the widest possible range of environmental conditions and types of submerged plant communities. A recording echosounder (Sitex-Honda, model HE-356, provided with a 50-kHz transducer with a cone beam of 50°) was used to obtain the tracings of the littoral vegetation profiles. The maximum chart speed (5.75 cm·min^{-1}) was used in combination with the lowest stable travelling speed to yield the best possible resolution. The speed conversion factors ranged from 6.8 to 10.3 cm of echosounder chart per 100 m of distance. Six echosounder transects, perpendicular to the depth contours, were run from the shoreline to the depth of disappearance of the submerged vegetation to obtain a reliable statistical representation of the height of the vegetation at each site. Where dense plant growth occurred, the power of the acoustic signal was increased in order to allow discrimination of the bottom. A mark was made on the chart and a buoy was dropped as the echosounder tracing passed over the macrophyte community selected for calibration. Subsequently, SCUBA divers harvested the aboveground vegetation in six random 0.09-m^2 quadrats within 1.5 m to either side of the buoy and at the same depth. The plants were kept refrigerated and rinsed free of detritus and animals, sorted by species, spun in a lettuce spin-dryer to remove excess water, and then weighed to the nearest 0.01 g.

As the heights of individual plants within a stand are usually normally distributed (Harper 1977; C. M. Duarte, in progress), the height of the stand sampled was estimated from the echosounder chart as the midheight between the tallest and the shortest trace within 1 m to either side of the point for each of the six echosounder tracings, and then the average of the six estimates was computed. Because of the wide cone beam of the transducer used (50°), the heights represented in the chart correspond to that of clusters of plants about 80 cm in diameter. Linear regression was used to establish the best equation to estimate the mean biomass, obtained from the six quadrats collected, from the average plant height obtained from the six echosounder transects.

Results and Discussion

The macrophyte beds sampled covered a wide range of biomass and vegetation height (Table 1) and included 22 species (Appendix). Fourteen of the 36 stands were monospecific (one species representing more than 90% of the biomass). The SCUBA estimates of biomass were much more variable than the echosounder estimates of height, the mean coefficient of variation for the SCUBA obtained biomass estimates being fivefold higher than that for echosounder estimates of vegetation height (Table 1). The standard deviations of direct biomass estimates increased with increasing biomass as

$$(1)\quad \text{SD biomass} = 25.1 + 0.30\,\text{biomass}$$

$$R^2 = 0.59;\ F = 47.6;\ P < 0.0001$$

where biomass is measured in grams fresh weight per square metre. The correlation between height estimates and their errors was not statistically significant ($R = 0.21$; $P > 0.05$). The equation of best fit to estimate biomass from vegetation height was

$$\text{Biomass} = 681.5 + 9.68\,\text{height}$$

$$R^2 = 0.55;\ F = 40;\ P < 0.0001;\ \text{SE}_{\text{est.}} = 831$$

where height is measured in centimetres. The relationship has a modest predictive power (Fig. 1), the standard error of the estimates being about twice that obtained from direct biomass estimates (average standard error of the biomass estimates is 505 g fresh wt.·m^{-2}). Equation 2 also results in different errors for different groups of plants (Fig. 1). No pattern was detected in the distribution of the residuals across lakes. The predictive power of the relationship between height and biomass is comparable with that reported by Maceina and Shireman (1980) who used different regression equations for sparse and dense vegetation, although their method derived for subtropical submerged macrophytes cannot be directly applied to the temperate stands used here. They found the relationship between echosounder-derived variables and biomass to vary

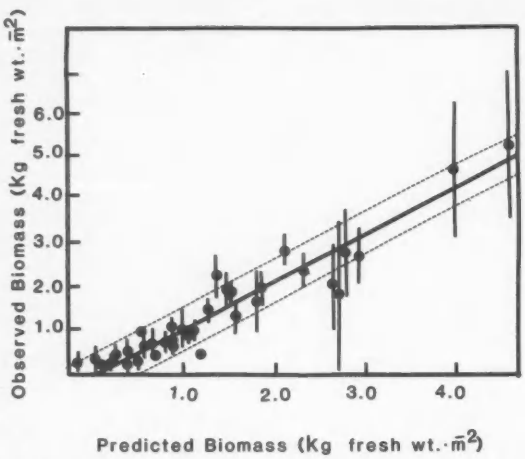

FIG. 2. Predicted biomass (Equation 3) versus SCUBA-obtained biomass. Vertical bars represent ± 1 SD for the SCUBA-obtained biomass estimates, and broken lines represent ± 1 SE for the predicted biomass.

among lakes (Maceina et al. 1984). The reason why a simpler regression (Equation 2) performs as well as their more complex models is probably because six replicates for both SCUBA-derived biomass and echosounder-derived height were used in the present study whereas their estimates were obtained from only a single sample per site. Yet, all these methods permit only rough approximations of the biomass of submerged vegetation.

The B/H of submerged vegetation varies between 500 and 5000 g fresh wt.·m^{-3}. Since the relationship between plant biomass and stand height is also dependent on B/H, the biomass estimates obtained from height alone cannot be precise. However, B/H values are a function of plant growth form (C. M. Duarte and J. Kalff, submitted), and this allows the division of macrophyte stands into three groups based on the growth form of the dominant species. The first class includes tall canopy-forming species that reach the surface to flower (e.g. *Myriophyllum*, Appendix) and has the smallest B/H values (430–1300 g fresh wt.·m^{-3}). Class 2 consists of short understory species that develop flowers that float to the surface attached by a short peduncle (e.g. *Utricularia*, Appendix) and presents intermediate B/H values ranging from 1700 to 3000 g fresh wt.·m^{-3}. Lastly, class 3 includes the species with submerged flowers (e.g. *Ceratophyllum, Rupia*, Appendix), species that lack flowers (e.g. *Isoetes, Chara*, Appendix), and those that, while able to form flowers, never do so in nature (e.g. *Egeria*, Appendix). Class 3 species show the highest B/H, ranging from 3500 to 5500 g fresh wt.·m^{-3}.

Differences in B/H and in growth form of established plant communities suggested a simple way to improve the accuracy of echosounder-derived biomass estimates. Consequently, the macrophyte stands were grouped by form, resulting in 18 class 1 stands, 13 class 2 stands, and 5 class 3 stands. Since the average B/H values for plants in classes 2 and 3 are about two and three times that of class 1, respectively, (C. M. Duarte and J. Kalff, submitted), we assigned form values from 1 to 3 to the stands depending on whether their dominant species belong to class 1, 2, or 3, respectively. The new equation that considers the relationship between B/H and growth form is

$$(3)\quad \text{Biomass} = -343 + 37.7\,\text{height}^3 + 953(\text{form}\cdot\text{height})$$
$$R^2 = 0.89;\ F = 126;\ SE_{est.} = 421.2$$

where biomass is measured in grams fresh weight per square metre and height in metres. The precision of this equation is not only much greater than that using height alone (Fig. 2), but the plant form classes contribute nearly as much to the variance explained as height (partial F for height^3 = 135; partial F for height·form = 116). The regression coefficient for form·height results in biomass estimates very close to the B/H characteristic of each growth class when the calculations for stands 1 m tall are performed. Using the new equation, the standard error of the biomass estimate is reduced by half (Equation 2) and it is not larger than the average standard deviation for the direct sampling (±425.8 g fresh wt.·m^{-2}). Thus, echosounder estimates that consider growth form as a second variable appear to yield as reliable biomass estimates as SCUBA techniques, particularly for biomass above 1320 g fresh wt.·m^{-2} (Fig. 2). The need to record growth form as a second variable is not cumbersome, since it can be noted from the echosounder traces, at least at the time of maximum biomass. Alternatively, glass bottom cylinders can be used to record the form of the vegetation at the time of echosounding. Where water turbidity prevents this, grab samplers can be used to characterize the growth form of the vegetation.

Direct harvesting is an expensive and time-consuming procedure (see Downing and Anderson 1985). Two SCUBA divers require 20 min on average to harvest the biomass of six replicate quadrats at a single depth. In contrast, six replicate echosounder transects require only 8–35 min to obtain biomass estimates for all depths, with the actual time required dependent on the littoral slope and the depth to which the plants grow. Additional advantages of the echosounder method are (1) a continuous record of the vegetation, rather than at discrete depths only, with the latter resulting in inaccuracies when the mean biomass values are estimated, (2) nondestructive sampling, which allows monitoring of the growth of stands over time and (3) simultaneous recording of other variables such as percent cover (Stant and Hanley 1985), volume occupied by the submerged vegetation, and littoral slope (Duarte and Kalff 1986), which influences macrophyte biomass.

The new method has several limitations which are relatively minor. It cannot be used in very shallow water, 40–70 cm being the minimum depth that could be recorded with the echosounder used in this study. Secondly, echosounders equipped with transducers similar to the one used here do not yield accurate estimates of vegetation under 20 cm in height. This limitation can be overcome with the use of high-precision transducers with higher frequencies (120–200 kHz) and smaller beam angles (8°); these modifications allow more accurate biomass estimates, particularly in very sparse vegetation and where the vegetation is as low as 10 cm. Lastly, when vegetation extends to the water surface, the canopy top does not appear on the tracings, and field notes must be taken to indicate where the height of the vegetation equals the water depth. A highly diverse vegetation may result in ambiguities in assigning a growth form class to stands. In our experience, most stands are dominated by species of a single growth form; most of the stands sampled in nine Québec lakes were monospecific. In an intensive survey of Lake Memphremagog (Québec–Vermont, Duarte and Kalff 1987), nine very diverse

stands containing plants from all three form classes were found, their B/H being remarkably constant at about 1800. However, the constancy of this B/H for very diverse stands is not to be taken for granted. Consequently, B/H values for mixed stands should be calculated by researchers working in lakes where highly diverse stands are common, and Equation 3 should be suitably modified to incorporate the new class. Since the error around SCUBA biomass estimates increases with biomass (Equation 1; Fig. 2), SCUBA techniques may be preferable to the use of echosounders when accurate estimates are required in lakes with very small macrophyte biomass. The present model (Equation 3) was developed for temperate zone lakes. As B/H increases with decreasing latitude (Duarte and Kalff 1987), recalibration will be necessary for the use of the method at lower latitudes.

In summary, the new model, based on both plant height and form (B/H), allows much more accurate estimation of submerged vegetation biomass than hitherto possible from simple echosounder measurements. This method allows for continuous estimates of underwater biomass with a precision similar to that obtained from cumbersome and time-consuming SCUBA harvesting.

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Appendix

Growth form classification of the macrophyte species encountered in this study.

Class 1	Class 2	Class 3
Myriophyllum exalbescens	*Cabomba caroliniana*	*Ceratophyllum demersum*
M. spicatum	*Heteranthera dubia*	*Chara* sp.
Potamogeton amplifolious	*Pomatogeton robinsii*	*Elodea canadensis*
P. crispus	*Saggitaria* sp.	*Isoetes lacustris*
P. gramineous	*Utricularia vulgaris*	*I. macrospora*
P. illinoensis	*Vallisneria americana*	*Najas flexilis*
P. perfoliatus		*Nitella* sp.
P. praelongus		
P. richardsonii		

Highly Specialized Nitrogen Metabolism in a Freshwater Phytoplankter, *Chrysochromulina breviturrita*

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Wehr, J. D., L. M. Brown, and K. O'Grady. 1987. Highly specialized nitrogen metabolism in a freshwater phytoplankter, *Chrysochromulina breviturrita*. Can. J. Fish. Aquat. Sci. 44: 736–742.

A field and laboratory culture study was carried out on the nitrogen metabolism of isolates of the freshwater phytoplankter *Chrysochromulina breviturrita* Nich. (Prymnesiophyceae). These were isolated from two different softwater lakes, one believed to be influenced by acidic precipitation (Cinder Lake) and another which was experimentally acidified with H_2SO_4 (Lake 302-South). The alga was able to utilize only NH_4^+ as an inorganic N source. A range of irradiances and molybdenum concentrations failed to induce NO_3^- utilization. Among 17 organic N compounds including amino acids, purines, and other amines, only urea plus Ni^{2+} as a cofactor would serve as the sole N source for this species. Nonetheless, growth rates in media supplied with urea were significantly less than with NH_4^+. Field data from Lake 302-S indicate that a predominance of NH_4^+ versus NO_3^- as the major inorganic N species may have favored the development of a *Chrysochromulina*-dominated community during August 1984. A detailed depth profile also indicated that a metalimnetic peak ($>20 \times 10^6$ cells/L) of this alga coincided with a distinct NH_4^+ depletion, which occurred at no other time during the year. Experiments with isolates of *C. breviturrita* and a *Nannochloris* sp. (Chlorophyceae) (~1 μm in diameter) from this community indicated that the former alga possessed a highly specialized N metabolism much like the Cinder Lake isolate. The *Nannochloris* sp. from the same environment grew on NO_3^- and NH_4^+ equally well. It is suggested that the specialized NH_4^+ utilization by *C. breviturrita* may itself influence the pH regime of poorly buffered waters through selective NH_4^+ uptake and H^+ generation.

Les auteurs ont étudié sur le terrain et en laboratoire le métabolisme de l'azote chez des isolats de l'organisme phytoplanctonique d'eau douce *Chrysochromulina breviturrita* Nich. (prymnésiophycées). Les organismes ont été prélevés dans deux lacs d'eau douce différents. Le premier, le lac Cinder, semble subir les effets des précipitations acides. Le second, le lac 302-sud, a été expérimentalement acidifié au H_2SO_4. Les algues ne pouvaient utiliser que le NH_4^+ comme source d'azote inorganique. L'utilisation d'une gamme variée d'irradiations et de diverses concentrations de molybdène n'a pas permis d'induire l'utilisation du NO_3^-. Des 17 composés azotés organiques utilisés, notamment des acides aminés, des purines et d'autres amines, seule l'urée (avec du Ni^{+2} comme co-facteur) était utilisée comme source d'azote par cette espèce. Les taux de croissance obtenus en milieux enrichis à l'urée étaient cependant significativement inférieurs à ceux obtenus en milieux enrichis au NH_4^+. Les données obtenues sur le terrain au lac 302-S indiquaient que la prédominance du NH_4^+ par rapport au NO_3^- comme principale substance azotée inorganique avait pu favoriser l'expansion, en août 1984, d'une communauté dominée par les *Chrysochromulina*. Un profil des profondeurs détaillé indiquait aussi l'existence d'un pic métalimnétique ($>20 \times 10^6$ cellules/L) de cette algue qui coïncidait avec une nette carence de NH_4^+, phénomène qui ne s'est produit à aucun autre moment de l'année. Des essais portant sur des isolats de *C. breviturrita* et de *Nannochloris* sp. (chlorophycées) d'environ 1 μm de diamètre prélevés de cette communauté ont montré que la première algue possédait un métabolisme fortement spécialisé de l'azote qui ressemblait beaucoup à celui noté pour l'isolat du lac Cinder. Les *Nannochloris* sp., qui provenaient du même environnement, utilisaient aussi bien le NO_3^- que le NH_4^+. Les auteurs émettent l'hypothèse que l'utilisation spécialisée du NH_4^+ par *C. breviturrita* peut en elle-même influer sur le régime du pH des eaux mal tamponnées de par une assimilation sélective du NH_4^+ et la production de H^+.

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While phosphorus is widely recognized as the nutrient which most commonly regulates algal productivity in lakes (Schindler 1977; Wetzel 1983), nitrogen may influence algal biomass in some systems and may also affect species composition (Kalff 1971; Liao and Lean 1978). Phytoplankton growth in Castle Lake, a mesooligotrophic lake in California, is limited at least during part of the year by N availability and regeneration (Axler et al. 1982). Several forms of N are usually present and are recycled in freshwaters. The cycling of ammonium (NH_4^+) is particularly rapid (a few hours), which indicates a close coupling between regeneration and assimilation (Brezonik 1972; Axler et al. 1981).

In oligotrophic, granitic basins, NH_4^+ inputs are frequently derived in large part from atmospheric sources such as gaseous forms and precipitation (Likens et al. 1977). In such systems, which are also influenced by acidic precipitation, inputs of NH_4^+ are retained much more strongly than nitrate (NO_3^-) (Galloway et al. 1983). This could be significant, particularly as NH_4^+ represents on average the second most abundant

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cation in acidic precipitation (Gorham et al. 1984). Further, it has been shown in whole-lake experiments that NH_4^+ utilization by phytoplankton may further acidify poorly buffered waters (Schindler et al. 1985).

Culture studies have indicated that nearly all algae can utilize either NO_3^-, NO_2^-, or NH_4^+ as a N source. The latter form is utilized most efficiently, as more oxidized forms must be reduced before being incorporated into organic N compounds (Healey 1973; Syrett 1981). Our interest in N metabolism arose while developing a dilute, defined culture medium for the freshwater phytoplankter *Chrysochromulina breviturrita* Nich. (Prymnesiophyceae) (Wehr et al. 1985). In nature, this organism "blooms" in softwater lakes influenced by acidic precipitation and is associated with characteristic sour or rotten cabbage odors (Nicholls et al. 1982). In preliminary culture experiments, we found that the alga could use NH_4^+, but apparently not NO_3^- or urea, as the sole N source (Wehr et al. 1985). In addition, cells grown over the short term (~ 10 d) in a NO_3^--based medium had significantly less chlorophyll *a* per cell than NH_4^+-grown cells. Utilization of NH_4^+, as shown previously (e.g. Pratt and Fong 1940; Brewer and Goldman 1976), also led to the acidification of the growth medium unless a pH buffer was used (Wehr et al. 1986).

Relatively few freshwater algal species have been shown to have distinct preferences for certain forms of N. The most widely studied group are the euglenoids, which typically occur in acidic waters. Many isolates will not utilize NO_3^-, but all can apparently substitute a wide variety of organic N compounds (especially amino acids) for NH_4^+ (Cramer and Myers 1952; Birdsey and Lynch 1962; Cook 1968; Kempner and Miller 1972). One marine cryptomonad, *Hemiselmis virescens*, has been shown to possess a similarly limited N metabolism (Antia et al. 1975). *Cyanidium caldarium*, which occurs in acidic hot springs, is also somewhat specialized, with some isolates which can and others which cannot use NO_3^- as a N source (Rigano et al. 1975), although a number of organic compounds will suffice. An unusual example of NO_3^- preference has also been described in two *Haematococcus* spp. (Proctor 1957), which was shown to be related to pH interactions with NH_4^+ toxicity (Stross 1963). This might be expected, particularly with the excessive N concentrations used in these early studies (millimolar range).

Although many algal species apparently "prefer" NH_4^+ over NO_3^- or organic N (Syrett 1981), we know of no studies in which neither NO_3^- nor an array of organic N compounds can supply the N needs of an alga. In the present study, we demonstrate in detail that *C. breviturrita* possesses an exceptionally specialized N metabolism and that this has particular importance when blooms are formed in acid-sensitive lakes.

Materials and Methods

Culture Experiments

Chrysochromulina breviturrita was originally collected in July 1982 using surface water (1 m depth) grab samples from Cinder Lake (78°51′W, 45°04′N), a moderately acidic, oligotrophic lake in south-central Ontario. It was isolated into unialgal, axenic culture and has been maintained since early 1984 in a chemically defined medium which approximates the chemistry of softwater lakes. Exhaustive electron microscope studies of this isolate (BT130) indicated that *C. breviturrita* was the only species present in culture. Complete details of isolation and the culture medium have been given previously (Wehr et al. 1985). Nearly all culture experiments were conducted using this isolate. In one experiment, two other phytoplankters were used. *Chrysochromulina breviturrita* (isolate BT138: identity determined by TEM) and *Nannochloris* sp. (isolate LRGT-1: ~1 μm in diameter) were both isolated from a sample collected from Lake 302-South in the Experimental Lakes Area (northwestern Ontario) in August 1984. Details of this site are given in the field program below. Both were rendered unialgal and axenic by methods described previously (Wehr et al. 1985). The plus-N medium routinely used for maintenance of cultures (Muskoka No.112) is identical to that used in previous studies (No. 42: Wehr and Brown 1985; Wehr et al.1985) except that half the N and P (50 μ*M* $(NH_4)H_2PO_4$) and only two vitamins (thiamine, B_{12}) were added. The water used for media was glass distilled and then passed through a cation-exchange column (Biorad AG50-8W, H^+ form) to remove trace NH_4^+-N. Experimental cultures were grown in acid-washed (10% HCl) 125-mL Erlenmeyer flasks containing 50 mL of medium. They received an irradiance of 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ on a 12:12 LD photoperiod ("cool-white" fluorescent lights) at 20°C, unless stated otherwise. In one experiment a range of irradiances ($10-200$ $\mu E \cdot m^{-2} \cdot s^{-1}$) were also tested. When nitrate (as $Ca(NO_3)_2$) or other N compounds were supplied, P was added as NaH_2PO_4. Organic N compounds (and NH_4^+ controls) were added after autoclaving the minus-N basal medium via filter sterilization (0.2-μm pore size). All amino acids (except glycine) used were in the L form. Experimental treatments were run in triplicate. Prior to a specific N treatment, cells were grown in a NH_4^+-N based medium (Muskoka No. 112) up to late exponential phase (~7–9 d). Approximately 80–90% of the medium (minus cells) was then aseptically aspirated off and replaced with an equivalent volume of N-deficient medium. Cells remained in this regime for approximately 3–4 d prior to inoculation into experimental conditions. This "starvation" was, however, probably insufficient to completely eliminate traces of NH_4^+-N being carried over with the inoculum. Cultures were inoculated to produce an initial density of between 2×10^3 and 5×10^3 cells/mL. Cells were counted using a Bio/Physics (model 6300A) laser-based particle counter which was calibrated daily with hemacytometer counts.

Field Program

In August 1984, a bloom with odor of *C. breviturrita* (plus *C. laurentiana* Kling: identified by TEM) occurred in the south basin of Lake 302 in the Experimental Lakes Area. Details of geography and bathymetry of this lake are given in Brunskill and Schindler (1971). The lake was divided in half in 1981 using a vinyl curtain and experimental acidification of the south basin with concentrated H_2SO_4 (electrolyte grade) began in 1982. Acidification procedures were similar to that described for Lake 223 (Schindler et al. 1980). Most of the HCO_3^- in the epilimnion of Lake 302-S was destroyed during the first year, while in subsequent years further acid was added to decrease the pH by about 0.3 units/yr (Schindler 1985). In 1984, annual pH averages in both the epilimnion and the whole lake were 5.6. Average total alkalinities in these two regimes were 16 and 4.5 μEq/L, respectively (M. A. Turner, Freshwater Institute, Winnipeg, Man., pers comm.).

Nitrogen chemistry and phytoplankton abundance in Lake

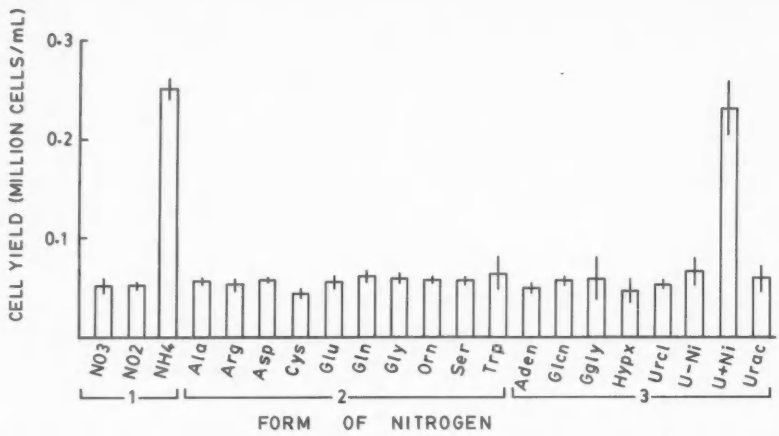

FIG. 1. Comparison of growth (expressed as cell yield) of *C.breviturrita* when supplied with 21 different N compounds as the sole N source. All N sources (group 1 = inorganic, group 2 = amino acids, group 3 = other organic forms) were supplied at a concentration of 50 μM. Results represent growth in experiments based on the first subculture only (n = 3 per treatment, error bars represent ± 1 SD). Ala = alanine, Arg = arginine, Asp = aspartic acid, Cys = cysteine, Glu = glutamate, Gln = glutamine, Gly = glycine, Orn = ornithine, Ser = serine, Trp = tryptophan, Aden = adenine, Glcn = glucosamine, Ggly = glycylglycine, Hypx = hypoxanthine, Urcl = uracil, U − Ni = urea minus Ni^{2+}, U + Ni = urea plus Ni^{2+}, Urac = uric acid.

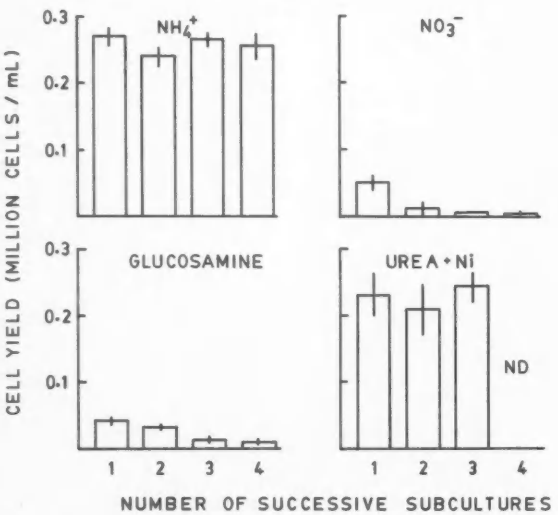

FIG. 2. Long-term growth and maintenance of *C. breviturrita* in media supplied with either NH_4^+, NO_3^-, glucosamine, or urea plus 1 μM) Ni^{2+}. N was supplied at a concentration of 50 μM. Each culture (n = 3) was sequentially transferred to an identical medium upon reaching the late exponential phase (~7 d for NH_4^+, NO_3^-, glucosamine; ~12–14 d for urea).

302-S were compared during the development of the bloom. In 1984, water was sampled at monthly intervals for chemical analysis at 1-, 4-, 6-, 7-, 8-, 9-, and 10-m depths using a peristaltic pump with polyethylene tubing at a station near the deepest part of the lake. The epilimnion was sampled weekly. Methods used for the analysis of NO_3^-, NH_4^+, and organic N follow those described in Stainton et al. (1977) and Linsey et al. (1985). Phytoplankton were also collected by an integrating sampler (Shearer 1978) where depths were divided into three strata: epilimnion, metalimnion, and hypolimnion. The depth of each stratum was based on temperature (epi-metalimnion) and light (meta-hypolimnion) profiles on a given date (J. A. Shearer, Freshwater Institute, Winnipeg, Man., pers. comm.). Samples were preserved using Lugol's iodine. Identification of *Chrysochromulina* spp. was carried out by D. L. Findlay (Freshwater Institute, Winnipeg, Man.) using light microscopy. Hence, *C. parva* was counted separately, but *C. breviturrita* and *C. laurentiana* were counted together as a single taxon. Details of routine preservation, counting, and enumeration methods as applied to Lake 223 samples (identical to 302-S procedures) are given in Findlay and Saesura (1980). Subsequent electron microscope (TEM) studies of plankton samples from Lake 302-S revealed the presence of both *C. breviturrita* and *C. laurentiana*.

Results

A comparison of *C. breviturrita* growth (expressed as final cell yield) in a NH_4^+-N medium with that observed with media containing 19 other inorganic and organic N compounds as the sole N source (Fig. 1) revealed a highly specialized N metabolism. All were supplied at 50 μM N concentrations. Only urea, when supplied with Ni^{2+} as a cofactor (1 μM), proved to support growth which was statistically equivalent to NH_4^+ controls (ANOVA, $p < 0.05$). When Ni^{2+} was not supplied, growth was not stimulated. Lower Ni^{2+} concentrations which have been tested (e.g. 0.1 μM) have resulted in intermediate yields. All other forms of N tested yielded significantly lower ($p < 0.05$) yields (approximately 20% that of controls on the first subculture into these treatments). The acidification of the

TABLE 1. Influence of irradiance (PAR: photosynthetically active radiation) and Mo concentration on cell yields of *C. breviturrita* (after 21 d) in media supplied with NO_3^--N, compared with NH_4^+-N controls. All N sources supplied at 50 μM (n = 3); ND = no data available.

Treatment	Cell yields (10^5 cells/mL) at irradiance ($\mu E \cdot m^{-2} \cdot s^{-1}$): 10	60	100	200
NO_3^- + 50 nM Mo	0.48 ± 0.05	0.71 ± 0.15	0.53 ± 0.17	0.55 ± 0.06
NO_3^- + 100 nM	ND	0.63 ± 0.04	ND	0.65 ± 0.01
NO_3^- + 1000 nM Mo	ND	0.64 ± 0.08	ND	0.47 ± 0.08
NH_4^+	0.73 ± 0.26	2.03 ± 0.16	2.10 ± 0.29	1.69 ± 0.14

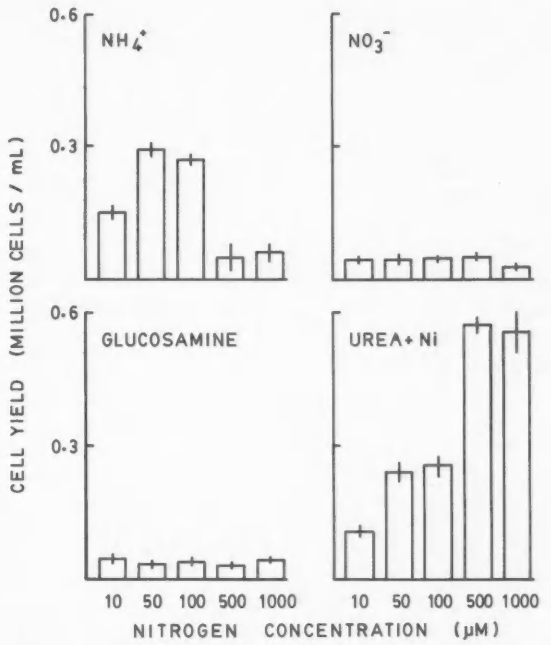

FIG. 3. Influence of N concentration on final cell yields of *C. breviturrita* with N supplied with either NH_4^+, NO_3^-, glucosamine, or urea (plus 1 μM Ni^{2+}) as the sole N source (n = 3, error bars represent ± 1 SD). Cultures were subcultured every 7 d (NH_4^+, NO_3^-, glucosamine) or 10–12 d (urea).

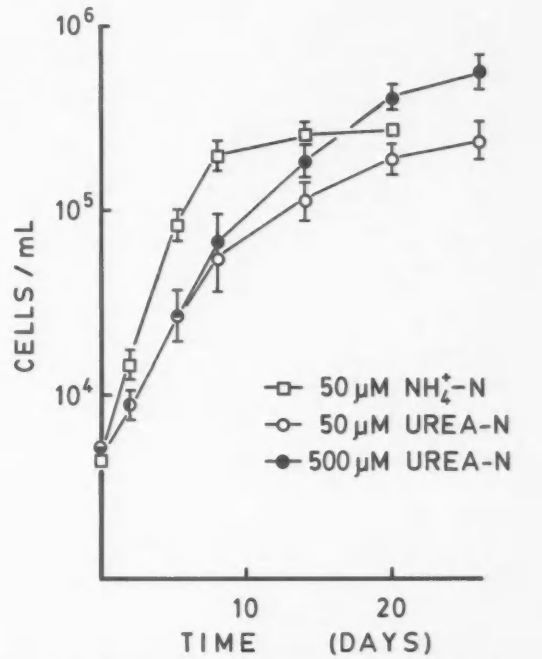

FIG. 4. Growth curves of *C.breviturrita* when supplied with 50 μM NH_4^+, 50 μM urea (plus Ni^{2+}), or 500 μM urea (plus Ni^{2+}). Each treatment was replicated three times (SD bars less than symbol size not shown).

medium was considerably less when *C. breviturrita* was grown on urea-N (pH 6.0–5.30) than on NH_4^+-N (pH 6.0–4.5). A decline in pH to 5.30 was not significantly different from that measured in a NO_3^--N based medium (to pH 5.26), where negligible growth was observed.

Long-term growth of *C. breviturrita*, when supplied with different N sources (Fig. 2), revealed that the low yields initially observed with NO_3^--N and glucosamine-N could not be maintained. After growth had reached late exponential phase, cells were transferred to fresh media containing the same N source. No evidence was found to indicate that the alga was capable of adapting over time (~ 40 d) to either NO_3^- or glucosamine utilization. Maximum yield declined to levels less than 5% of controls. In contrast, urea proved to be capable of supporting growth upon the first subculture and maintaining similar yields over successive transfers.

An attempt was made to induce NO_3^- utilization by increasing the irradiance (stimulation of nitrate reductase) and Mo concentrations (enzyme cofactor) (Table 1). No increase in cell yield was observed in the NO_3^- medium at 60–200 $\mu E \cdot m^{-2} \cdot s^{-1}$. Lower light resulted in reduced yield, which was also observed with controls. Greater Mo concentrations also failed to induce NO_3^- utilization, even in combination with greater irradiance.

The same N compounds (NO_3^-, NH_4^+, urea, glucosamine) which were tested for long-term effects (at 50 μM) were also compared over a broad concentration range (Fig. 3), from 10 to 1000 μM. No significant increases were observed in the growth of *C. breviturrita* ($p > 0.05$) for either NO_3^- or glucosamine-N. At 1000 μM NO_3^--N, yield decreased significantly (~40%). The maximum yield in the NH_4^+-N treatments

TABLE 2. Changes in densities of *Chrysochromulina* spp. (*C. breviturrita* and *C. laurentiana*) in Lake 302-S in epilimnion ("epi") and metalimnion ("meta") strata after the addition of H_2SO_4 in 1982.

				Cell density (10^6 cells/L)					
		n		$\bar{x}$		SD		Maximum	
Treatment	Year	epi	meta	epi	meta	epi	meta	epi	meta
Pre-acid	1981	16	7	0.10	0.13	0.07	0.17	0.28	0.46
$+H_2SO_4$	1982	14	10	0.24	0.12	0.25	0.18	0.91	0.64
	1983	13	8	0.44	0.44	0.57	0.75	1.6	2.1
	1984	15	9	2.4	8.6	3.1	6.5	8.9	20.4

was observed between 50 and 100 μM, with an apparent toxicity at concentrations >500 μM. In contrast, yields more than doubled (2.47×10^5 vs. 5.73×10^5 cells/mL) in response to increased urea concentrations between 100 and 500 μM. These yields are the greatest so far achieved in culture for *C. breviturrita*. However, consideration of the entire growth curves for urea treatments (Fig. 4) clearly shows that maximum growth rates of this alga in urea-based media (e.g. 50 μM: 0.46 ± 0.067 divisions/d) are significantly less ($p < 0.05$) than in NH_4^+-N (50 μM: 0.78 ± 0.066 divisions/d). Maximum cell numbers are reached only after about 25 d, as compared with about 8–10 d with NH_4^+-N.

The development of greater populations of *C. breviturrita* (plus *C. laurentiana*) in Lake 302-S was observed following the addition of H_2SO_4 (Table 2). Maximum densities in the epilimnion and metalimnion prior to the addition of acid were 0.28×10^6 and 0.46×10^6 cells/L, respectively. Although standard deviations of annual averages frequently reached or exceeded 100% of the mean, these average densities also followed an increasing trend over this period. By 1984, populations in each stratum had increased more than 30-fold, resulting in the symptomatic bloom with "sour" or "rotten" cabbage odors in August. During the entire 1984 sampling period, concentrations of NH_4^+-N were nearly always greater than NO_3^--N, regardless of depth (G. A. Linsey, Freshwater Institute, Winnipeg, Man., unpubl. data). Densities of *Chrysochromulina* spp. during 1984 increased markedly after the spring turnover (late March – early April); epilimnion densities increased by a factor of five, while in the metalimnion, cell densities increased 20-fold. A detailed comparison of late spring and late summer populations with the N regime (Fig. 5) indicates that with this metalimnetic algal maximum there was a distinct depletion of NH_4^+-N. NO_3^--N concentrations, however, followed no obvious pattern in relation to depth or algal densities.

As a follow-up to these observations, *C. breviturrita* was isolated from a sample collected from Lake 302-S during the bloom. *Nannochloris* sp. (Chlorophyceae) was also isolated and is considered here for comparison. The *Nannochloris* sp. was confirmed to be a member of the Chlorophyceae by chlorophyll and carotenoid analyses and electron microscopy of sectioned material (unpublished data). Both axenic isolates were tested for growth in media that had either NO_3^- or NH_4^+ (50 μM) as the sole N source. Like the Cinder Lake isolate, *C. breviturrita* from L302-S was apparently unable to utilize NO_3^-. Upon first subculture the cell yield of this isolate was $0.33 (\pm 0.19) \times 10^5$ cells/mL versus $1.92 (\pm 0.10) \times 10^5$ cells/mL in a NH_4^+-based medium. Further subcultures in this medium have proven that this isolate also cannot be maintained with NO_3^--N only, substantiating earlier results (Fig. 2). In contrast, the *Nannochloris* sp. isolated from the same environment on the same date grew equally well on either form of N. The yield of this picoplankter, when using NO_3^--N, was $1.11 (\pm 0.13) \times 10^7$ cells/mL and with NH_4^+ was $1.21 (\pm 0.32) \times 10^7$ cells/mL (values not significantly different, $p < 0.05$).

Discussion

The present evidence from culture experiments (Table 1; Fig. 1–3) confirms earlier suggestions that *C. breviturrita* has an extremely specialized N metabolism. The fact that this alga may occur in massive numbers ("blooms") as the dominant alga in certain softwater lakes (Nicholls et al. 1982) is all the more remarkable and suggests that it is still a highly successful competitor. A restricted N metabolism has been demonstrated for euglenoids and other algal taxa from highly acidic environments (pH < 4.0) (Cook 1968; Rigano et al. 1975). However, none of these are as specific in their requirements as *C. breviturrita* and none are known from pelagic, planktonic communities in lakes. A few softwater phytoplankton species, such as the desmid *Arthrodesmus*, have recently been shown to utilize NO_3^-, urea, and in some cases one or more amino acids equally well (Vieira and Klaveness 1985). At present, little or nothing is known of the N requirements of other freshwater *Chrysochromulina* spp. (e.g. *C. parva*), although several marine species grow equally well on NO_3^- and NH_4^+, and can utilize at least some amino acids (Pintner and Provasoli 1968; Carpenter et al. 1972). The inability of *C. breviturrita* to grow successfully at NH_4^+ concentrations greater than 100 μM (Fig. 3) may suggest an extreme sensitivity to ammonia toxicity (i.e. NH_4^+), which would be present at less than 0.05% (25 nM), given the pH, ionic strength, and temperature regime in our experiments (Messer et al. 1984).

It is clear that NO_3^- will not serve as a N source for *C. breviturrita*. Light stimulation and Mo, factors well established to stimulate assimilation (Syrett 1981), had no effect (Table 1). Further, there was no evidence that the alga could adapt its metabolism if given sufficient time (Fig. 2). Some experimental evidence suggests that NO_3^- limitation may occur in marine phytoplankton due to unfavorable SO_4^{2-} to MoO_4^{2-} ratios (a competitive inhibition) exceeding $\sim 1 \times 10^5$ (Howarth and Cole 1985). In our experiments, control treatments had a ratio of $\sim 3 \times 10^3$ (Wehr et al. 1985), and elevated Mo concentrations (S:Mo ratios as low as 1.6×10^2) had no positive effect. The only form other than NH_4^+ that was utilized was urea and only in conjunction with Ni^{2+}. Such results suggest that the enzyme urease is present (Rees and

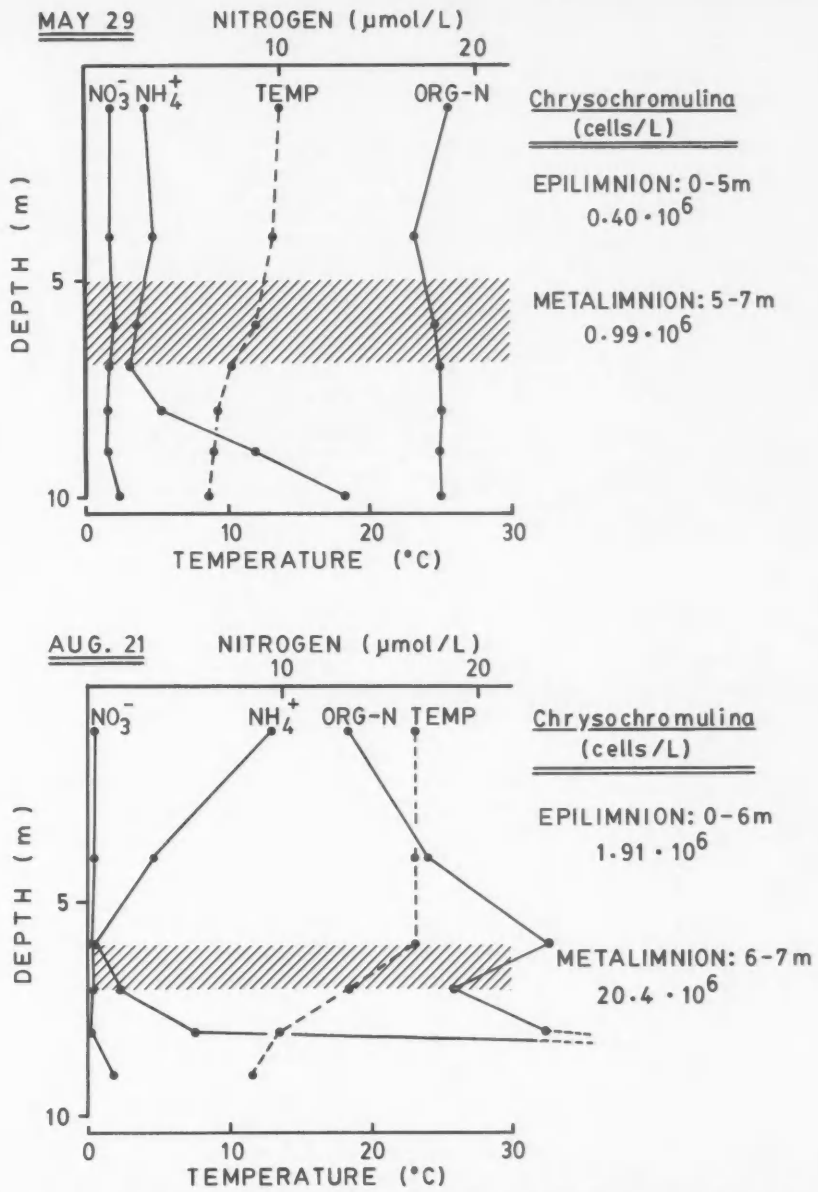

FIG. 5. Vertical profiles of filtrable NO_3^-, NH_4^+, and organic N concentrations as well as temperature in Lake 302-S during May 29 and August 21, 1984, before and during a *Chrysochromulina* spp. bloom, respectively. Algal densities indicated for two integrated strata were measured immediately prior to the dates of the water chemistries.

Bekheet 1982; Eskew et al. 1983). However, this N source, while capable of supporting a considerable biomass, is apparently much less efficiently utilized than NH_4^+, judging from the significantly lower exponential growth rates (Fig. 4). Although specific urea concentrations in Precambrian Shield lakes subject to acidic precipitation are lacking, it would seem unlikely that this form of N is of importance in the ecology of *C. breviturrita*.

The predominance of NH_4^+-N over NO_3^--N and NO_2^--N in experimentally acidified Lake 302-S may have been one factor which favored the marked increase of *C. breviturrita* in 1984. In the first 2 yr of acidification (1982–83), NO_3^- tended to be the predominant inorganic N species in the euphotic zone (~0–7 m) during spring and summer (Linsey et al. 1985). By 1984, when *C. breviturrita* increased markedly, the N regime changed and NH_4^+ dominated throughout most of the year,

including May through August (see also Fig. 5). By late August 1984, a dense metalimnetic maximum of cells coincided with the depletion of NH_4^+ between 6 and 7 m in depth.

Considering the ability of *C. breviturrita* to develop (often abrupt) blooms (Nicholls et al. 1982), it may perhaps be expected that selective NH_4^+ utilization in a specific localized stratum (e.g. metalimnetic peak) could lead to short-term H^+ generation and further acidification. Broad surveys of water chemistry data (Linsey et al. 1985; G. A. Linsey, pers. comm.), however, indicate only slight pH differences with depth during periods of maximum cell numbers. It remains to be seen, however, possibly by the use of in situ experiments, whether such organism-mediated changes may occur.

Acknowledgements

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Nitrification: A Significant Cause of Oxygen Depletion Under Winter Ice

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Changes in concentrations of ammonium, nitrite, nitrate, and oxygen suggested the occurrence of significant nitrification throughout the water column of mesotrophic Lake St. George, Ontario, during the winter months from 1976 to 1984. The existence of nitrapyrin- and acetylene-sensitive ^{14}C-bicarbonate incorporation confirmed that bacterial nitrification occurred. During late January to early March (water temperature 2–3°C), nitrification occurred at an average rate of about 13 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$ for the years studied. Numbers of detectable nitrifying bacteria appeared too low (by 2 to 4 orders of magnitude) to account for the observed activity. The nitrification activity observed would result in average oxygen consumption amounting to 71% of the observed oxygen depletion. This shows that winter nitrification can be an important factor in promoting oxygen depletion and possibly winter-kill of fish.

Des changements dans les concentrations d'ammonium, de nitrites, de nitrates et d'oxygène suggèrent qu'il s'est produit une importante réaction de nitrification dans toute la colonne d'eau du lac St. George, un lac mésotrophe de l'Ontario, durant les mois d'hiver de 1976 à 1984. La présence d'incorporations de bicarbonate-C^{14} sensibles à la nitrapyrine et à l'acétylène confirme qu'il y a effectivement eu nitrification bactérienne. Durant la période qui s'étend de la fin de janvier au début de mars de chaque année étudiée (température de l'eau : 2 à 3°C), la vitesse moyenne de nitrification était d'environ 13 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$. Le nombre de bactéries nitrifiantes énuméré était de deux à quatre fois trop faible pour expliquer l'activité observée. On a calculé que l'épuisement de l'oxygène observé serait attribuable à 71% à la consommation moyenne d'oxygène qui résulte de la nitrification. Cela démontre que la nitrification hivernale peut être un important facteur d'épuisement de l'oxygène et pourrait expliquer la mortalité des poissons en hiver.

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Nitrification (the oxidation of ammonium and nitrite to nitrate) has been studied intensively in soils (Schmidt 1982) and in both freshwater and marine sediments (Billen 1976; Jones and Simon 1981). Significant nitrification can also occur in the aerobic hypolimnia of lakes, based on evidence from mass balance of inorganic nitrogen species (Christophi et al. 1981; Verdouw and Dekkers 1982; Takahashi et al. 1982; Hall and Jeffries 1984) and measurements of the incorporation of radioactive bicarbonate in the presence and absence of the nitrification inhibitor nitrapyrin (Billen 1976; Somville 1978; Vincent and Downes 1981; Vincent et al. 1981; Hall 1982). Evidence for nitrification has come also from studies of oxygen depletion rates (Wezernak and Gannon 1967), most probable number determinations of nitrifying bacteria (Christophi et al. 1979; Takahashi et al. 1982), and immunofluorescence studies (Stanley et al. 1979; Ward 1984).

There appears to be little information on nitrification in the water column of lakes in winter under ice, although unpublished data for Wintergreen Lake, Michigan, presented without comment (Wetzel 1983), suggests significant conversion of ammonium to nitrate during the winter months. Such a conversion also occurred during winter in the Bay of Quinte, Ontario, and in untreated limnocorrals in the same Bay (Liao and Lean 1978).

In the present paper we report evidence for significant nitrification under winter ice and suggest that this process can be a major factor in oxygen depletion and possibly winter-kill of fish.

Methods

Lake St. George (43°57′30″N, 79°25′30″W) is 2 km east of Oak Ridges and about 32 km north of Toronto, Ontario. Located on a height of land with no major inflow, its location minimizes pollution from upstream sources. It is a mesotrophic double-basin lake (10.3 ha) and is described by Knowles (1979), Knowles et al. (1981), McQueen and Lean (1983), McQueen et al. (1984), and Wolfe (1979). The present study was carried out in the 5.88-ha eastern basin of the lake which has a maximum depth of 17 m. The lake was selected because of its pronounced seasonal changes in the forms of nitrogen

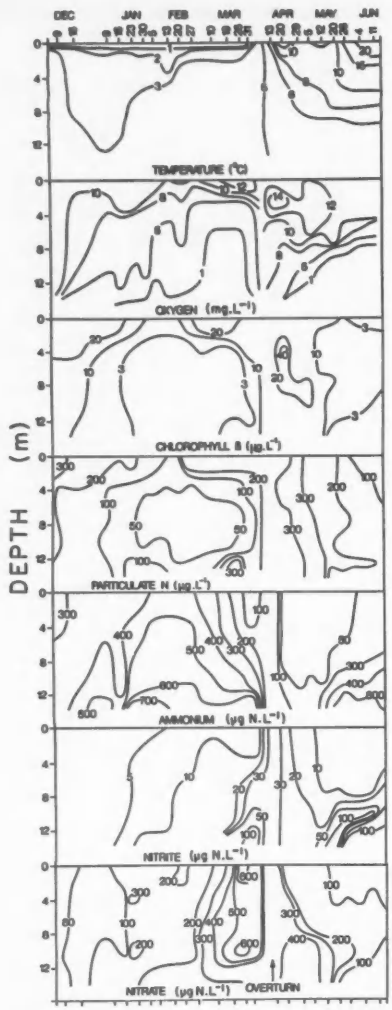

FIG. 1. Isopleths for temperature, oxygen, chlorophyll, particulate N, ammonium, nitrate, and nitrite for Lake St. George during the winter months of 1975–76.

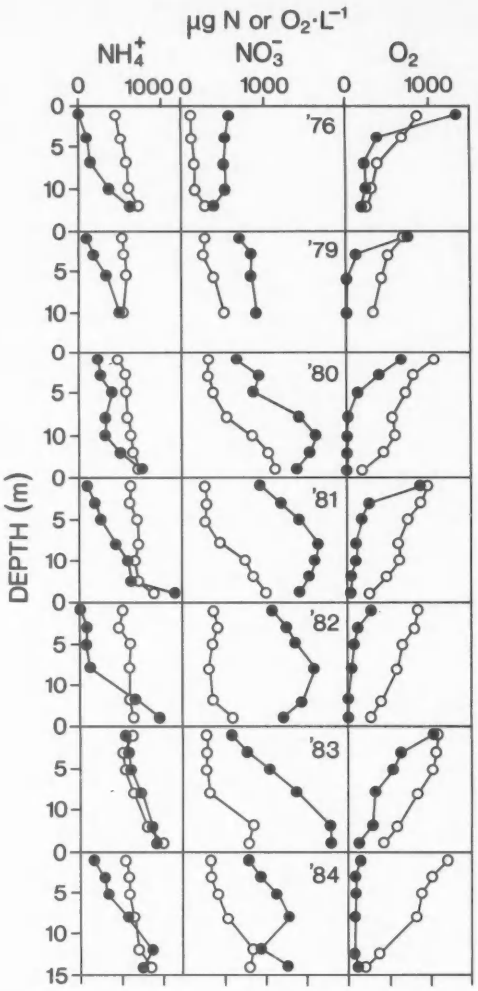

FIG. 2. Concentrations of NH_4^+-N, NO_3^--N, and O_2 in Lake St. George at the first (○) and second (●) sampling dates (given in Table 2) for winters between 1976 and 1984.

(Knowles et al. 1981). Surface nitrate concentrations range from near zero to 800 µg $N \cdot L^{-1}$.

Water samples were collected through the ice (0.3–0.4 m thick) using a 3-L Van Dorn water sampler or through tygon tubing connected to a peristaltic pump. Temperature and oxygen concentrations were measured using a Yellow Springs Instrument probe (model 54A). The presence of sulfide interfered, and in such cases, oxygen concentration was assumed to be zero. Samples were processed and analyzed for ammonium, nitrite, nitrate, and nitrous oxide as described by Knowles et al. (1981).

For bottle incubation experiments, 300-mL BOD bottles were filled with lake water from the appropriate depth. To avoid changes in dissolved gas concentrations, the sampler tube was kept at the bottom of the BOD bottle which was allowed to overflow for 10–15 s. Nitrification was assayed as acetylene-sensitive (Hynes and Knowles 1978) or nitrapyrin-sensitive dark incorporation of ^{14}C-bicarbonate (Billen 1976) and denitrification as the production of nitrous oxide in the presence of acetylene to inhibit reduction of nitrous oxide (Yoshinari and Knowles 1976; Knowles 1979). Where desired, nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) dissolved in dimethyl sulfoxide (DMSO) was added to give a final nitrapyrin concentration of 20 mg $\cdot L^{-1}$. Acetylene (25 mL) was added by syringe to each bottle through a rubber stopper, temporarily displacing water into a second syringe until all the acetylene had dissolved. Thus, the oxygen concentration was not altered. ^{14}C-bicarbonate (Amersham) was added to a final concentration of 150 000 (1980–82) and 700 000 (1983) dpm $\cdot mL^{-1}$. Drying out of the bottle stoppers was carefully avoided to prevent gas exchange. Bottles were incubated in the dark at close to in situ temperatures.

TABLE 1. Volumetric information and inorganic nitrogen data for two samplings from Lake St. George during the winter of 1982.

Depth (m) (sample layer)	Volume (10^6 L)	Jan. 24		Mar. 18		Rate[b]
		μg N·L^{-1}	kg N[a]	μg N·L^{-1}	kg N[a]	μg N·L^{-1}·d^{-1}
			Ammonium-N			
1 (0–2)	89.86	503	45.20	40	3.59	11.57
3 (2–4)	70.72	490	34.65	70	4.95	10.50
5 (4–7)	67.91	610	41.25	70	4.75	13.50
8 (7–10)	33.72	600	20.23	110	3.71	12.25
12 (10–13)	21.84	620	13.54	640	13.98	−0.50
14 (13–15)	8.56	640	5.48	960	8.21	−8.00
Mean		577.2		315.0		6.55
Total	292.61		160.53		39.20	
			Nitrite-N			
1 (0–2)	89.86	0	0.00	10	0.90	0.25
3 (2–4)	70.72	0	0.00	40	2.83	1.00
5 (4–7)	67.91	10	0.68	30	2.04	0.50
8 (7–10)	33.72	10	0.34	80	2.70	1.75
12 (10–13)	21.84	10	0.21	60	1.31	1.25
14 (13–15)	8.56	20	0.17	180	1.54	4.00
Mean		8.3		66.7		1.46
Total	292.61		1.41		11.31	
			Nitrate-N			
1 (0–2)	89.86	380	34.15	1090	97.95	17.75
3 (2–4)	70.72	410	28.99	1230	86.99	20.50
5 (4–7)	67.91	350	23.77	1380	93.71	25.75
8 (7–10)	33.72	340	11.46	1600	53.95	31.50
12 (10–13)	21.84	380	8.30	1440	31.45	26.50
14 (13–15)	8.56	640	5.48	1210	10.36	14.25
Mean		416.7		1325.0		22.71
Total	292.61		112.15		374.41	

[a]Data for kg N per layer obtained from volume × μg N·L^{-1}.
[b]Rate calculated from (change in concentration)/(interval in days).

TABLE 2. Rates of consumption of NH_4^+-N and O_2 and of production of (NO_2^- + NO_3^-)-N in situ in Lake St. George during winter months (μg N or O_2·L^{-1}·d^{-1}).[a]

Measure	Feb. 10 to Mar. 30, 1976	Jan. 31 to Mar. 20, 1979	Jan. 24 to Mar. 18, 1980	Jan. 14 to Mar. 24, 1981	Jan. 13 to Feb. 22, 1982	Jan. 09 to Feb. 15, 1983	Jan. 10 to Mar. 13, 1984	Mean
ΔNH_4^+	7.69	5.92	4.03	5.47	10.37	−0.24	3.56	5.26
$\Delta(NO_2^-$ + (NO_3^-)	8.51	9.25[b]	10.45	13.31	23.25	17.52	9.57	13.12
Calculated BOD_N[c]	36.80	40.00	45.20	57.60	100.70	75.90	41.40	56.80
Observed ΔO_2	−20.00	50.00	86.00	62.00	140.00	77.00	134.00	75.60
BOD_N ΔO_2 (%)	—	80.00	52.50	92.90	71.90	98.60	30.90	71.20[d]

[a]Mass balances calculated for the whole lake from concentration data of Fig. 1 and volumetric data in the depth range 0–15 m, except for 1976 (0–14 m) and 1979 (0–10 m), as exemplified by Table 1.
[b]NO_2^- data were not available for this year.
[c]Calculated O_2 consumption associated with the production of NO_2^- and NO_3^- assuming the factor of Wezernak and Gannon (1967): BOD_N = 4.33 nitrified-N.
[d]1976 data were not included in this calculation.

After appropriate intervals, oxygen was determined using a stirred probe with an Orbisphere oxygen meter. Chemical analyses were done as described above except that ammonium could not be analyzed in nitrapyrin treatments due to DMSO interference. Incorporation of ^{14}C during the first 0.2–1.8 d was determined by filtering (0.2-μm Nuclepore (for 1980–82) and 0.45-μm Sartorius 11306 (for 1983) membrane filters) 50 mL of sample. Each Nuclepore filter was acidified with 0.05

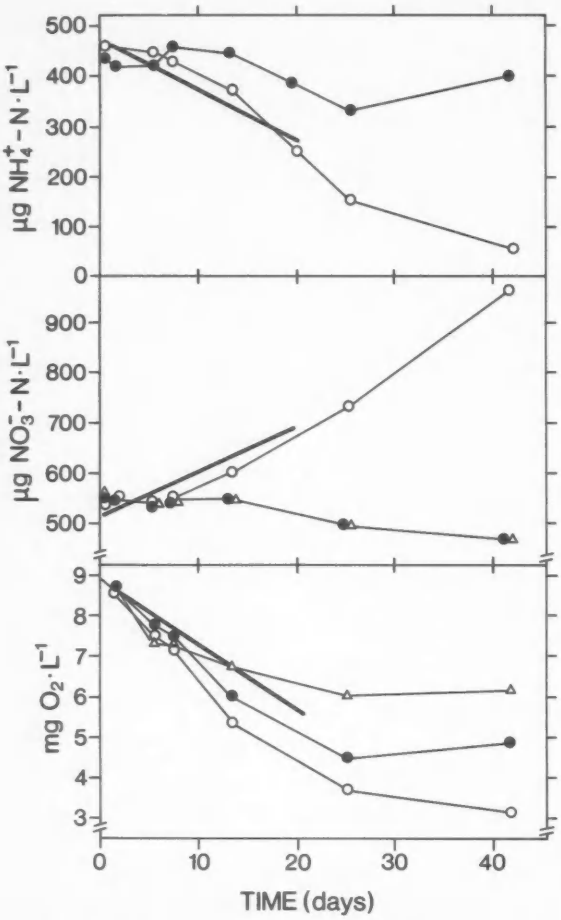

FIG. 3. Changes in concentrations of NH_4^+-N, NO_3^--N, and O_2 in samples of water (2-m depth) incubated in the absence (○) and in the presence of acetylene (●) or nitrapyrin ( ). The thick line shows changes in concentrations which occurred in the lake (1980) over the period indicated. Data for NH_4^+-N in the nitrapyrin treatment are not shown, since the DMSO solvent interfered with NH_4^+ analysis.

mL of glacial acetic acid and left to stand overnight in a fume hood and then dissolved with 10:1 methylene chloride – ethanolamine before addition of PCS fluor (Amersham) for liquid scintillation counting. Sartorius filters were acidified with 1 mL of 0.5 *M* H_2SO_4 for 2 h and then counted as above. Dissolved nitrous oxide was determined as described by Chan and Knowles (1979).

Nitrifying bacteria were enumerated by most probable number (MPN) methods as described by Alexander and Clark (1965) with an incubation period of 6 wk. Denitrifiers were enumerated by MPN essentially as described by Tiedje (1982) using Difco nutrient broth supplemented with 5 m*M* nitrate (final concentration).

Nitrapyrin (N-Serve) reagent grade (>99%) was a gift from Dow Chemical of Canada Ltd., Sarnia, Ontario; gases were from Union Carbide Canada, and other chemicals were of reagent grade.

Results

During routine monitoring of Lake St. George in 1975–76, it seemed that ammonium was being converted to nitrate during the February–March period. Isopleths through the winter period when gas exchange with the atmosphere is eliminated, and into spring overturn, show the period of oxygen depletion in the deep water and changes in chlorophyll, as well as the forms of nitrogen (Fig. 1). Winter began with a concentration of ammonium at about 300 throughout the water column increasing to greater than 500 $\mu g\ N \cdot L^{-1}$. From mid-December to 1 February, particulate nitrogen (and chlorophyll) declined, while nitrate and nitrite increased little. Consistent with the view that dissolved organic nitrogen is refractory, changes in the concentration of this form of nitrogen were trivial (data not shown).

During February and March the process of nitrification became what will be shown to be the most significant metabolic event occurring in the lake. Changes in particulate nitrogen were small and confined to the uppermost layers of the lake. Nitrite increased in the oxygen-poor deeper waters but ammonium decreased from roughly 500 to 150 $\mu g\ N \cdot L^{-1}$ in 60 d, while nitrate concentration showed a corresponding increase. Since changes in the other forms of nitrogen were small during the February period, only nitrate and ammonium concentrations need to be considered. (Raw data for this and subsequent years are provided in a series of data reports: Lean, McQueen, and Knowles. Water chemistry analysis for Lake St. George, Ontario. Canada Centre for Inland Waters Publ., in prep.)

This pattern of progressively decreasing concentrations of ammonium and oxygen and a simultaneous increase in nitrate concentrations was observed most years between 1976 and 1984 (Fig. 2). Nitrite levels also generally increased with time to maximum concentrations of 100–200 $\mu g\ N \cdot L^{-1}$ (data not shown). Oxygen was low and decreased with depth, and the bottom waters frequently became depleted towards the end of March (Fig. 1 and 2). Possibly due to this oxygen depletion, there was evidence of an input of ammonium from the sediment into the bottom of the water column with concentrations decreasing towards the surface (Fig. 1 and 2).

Mass balances of ammonium, nitrite, and nitrate nitrogen and oxygen were calculated by trapezoidal integration for the whole lake basin based on data for concentration (Fig. 2) and volume per layer (ice volume was ignored). As exemplified by Table 1 for 1982 data, there was generally a very significant decrease in whole lake total ammonium-nitrogen (121 kg of N for 1982) and a very significant increase in nitrate (262 kg of N for 1982). For 1982 also, the average rates of decrease in ammonium concentration and of increase in nitrite and nitrate concentrations were 6.55, 1.46, and 22.71 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$, respectively (Table 1, rightmost column).

For all the years investigated, and based on whole lake mass balance changes (as illustrated in Table 1), the rate of change in concentration of nitrogen oxides ($NO_2^- + NO_3^-$) was much greater than that of ammonium (Table 2), presumably due to an ammonium flux from the sediment or to mineralization of particulate nitrogen. The oxygen consumption associated with the oxidation of ammonium and nitrite to nitrate by nitrifying bacteria is 4.33 $\mu g\ O_2 \cdot \mu g\ N^{-1}$ oxidized through nitrite to nitrate (determined experimentally by Wezernak and Gannon 1967). Using this factor, it is clear that the oxygen demand of

TABLE 3. Summary of nitrapyrin-sensitive ^{14}C-bicarbonate incorporation experiments (0–0.21 or 1.8-d rates) giving initial concentrations of nitrate, nitrite, ammonium, and total particulate nitrogen ($\mu g\ N \cdot L^{-1}$) as well as oxygen ($mg \cdot L^{-1}$).

Date	Depth (m)	Initial concentration					Nitrification rate ($\mu g\ N \cdot L^{-1} \cdot d^{-1}$)
		NO_3^-	NO_2^-	NH_4^+	TPN	O_2	
Feb. 27, 1980	2	540	9	467	290	9.3	22.8
	8	1040	14	416	120	4.5	10.8
	14	1560	44	631	216	2.6	27.0
Mar. 6, 1980	2	550	5	390	82	8.1	12.2
	14	1570	7	512	78	2.0	13.1
Feb. 24, 1982	2	1230	36	65[a]	70	1.3	4.5
	8	1680	75	106[a]	67	0.3	7.7
	14	1400	183	858[a]	110	0.3	2.1
Mar. 3, 1982	2	1191	8	39[a]	69	0.8	1.3
	2[b]	1220	7	30[a]	60	6.8	1.7
Feb. 9, 1983	2[b]	510	20	490	110	10.2	10.5
	14[c]	1700	80	760	76	10.3	11.4

[a]Since ammonium was low at some depths, 300 $\mu g\ N \cdot L^{-1}$ was added to all samples indicated.
[b]The sample was filtered to remove zooplankton and oxygen was added.
[c]The sample was not filtered but it received oxygen.

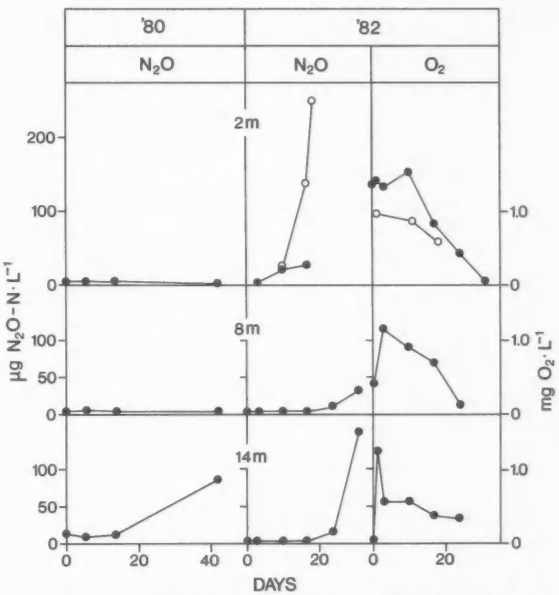

FIG. 4. Production of N_2O and consumption of O_2 in the presence of acetylene at 1.5 (○) and 3.3 (●) m*M*. There was no N_2O production in the absence of acetylene or in the presence of nitrapyrin.

the observed nitrification (BOD_N) is frequently a large percentage of the observed oxygen disappearance in the lake (Table 2), ranging from 30.9 to 98.6% (mean 71.2%). The actual rate of production of nitrite plus nitrate for the whole lake ranged from 8.51 to 23.25 (mean 13.12) $\mu g\ N \cdot L^{-1} \cdot d^{-1}$. The rate of increase in total mass of inorganic nitrogen during the periods studied ranged from 0.24 to 5.19 (mean 2.28) $kg\ N \cdot d^{-1}$, which translates to areal rates of 4.06–88.3 (mean 38.81) $mg\ N \cdot m^{-2} \cdot d^{-1}$. Over the total lake basin volume of 2.95×10^5 m^3, this represents a mean rate of increase of 7.73 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$, that is, approximately one half of the observed nitrification rate.

Nitrification was observed in water samples (2-m depth) incubated in bottles in the dark and at in situ temperature (Fig. 3). The accumulation of nitrate was inhibited by either nitrapyrin or acetylene, which also partially inhibited the consumption of oxygen. Acetylene also inhibited the disappearance of ammonium (Fig. 3). The rate of production of nitrate during the period 5–40 d was 13.3 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$.

Nitrapyrin- and acetylene-sensitive dark incorporation of ^{14}C-bicarbonate in samples (2-, 8-, and 14-m depth) of water incubated at in situ temperature was converted to nitrification rate using the ratio mg C fixed/mg N oxidized = 0.102 (Christofi et al. 1981). Nitrapyrin-sensitive ^{14}C-bicarbonate incorporation measurements were restricted to incubation periods of less than 2 d. The 12 experiments that were conducted (Table 3) provide estimates of nitrification in the range 1.3–27.0 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$, and thus they are similar to those obtained above (Table 2) by mass balance. The observed rates decreased with increasing incubation times over the first 5 d. Acetylene was not as effective as nitrapyrin in inhibiting ^{14}C-bicarbonate incorporation (data not shown). Nitrapyrin may inhibit other CO_2-consuming processes (Topp and Knowles 1982; Ward et al. 1982). There are simple problems of very low rates of incorporation, particularly in hardwater lakes such as Lake St. George (if the filters are not adequately decontaminated, any "incorporation" is obscured). DMSO controls also showed some inhibition over samples with no DMSO. It was, however, noteworthy that the nitrapyrin-sensitive dark ^{14}C-bicarbonate assimilation was only about 25% of total incorporation at 2 m but increased to near 50% at 8 m and 70% at 14 m. This no doubt represents the relative contribution of nitrification to oxygen losses as well.

In view of the appreciable nitrate levels and the frequently low oxygen concentrations, some assays for denitrification were carried out by incubating water samples containing acetylene. Denitrification, indicated by an accumulation of nitrous oxide, occurred only after about 3–16 d of incubation when the dissolved oxygen concentrations were in the range 0.3–0.9 $mg\ O_2 \cdot L^{-1}$ (Fig. 4). The potential denitrification rates occurring during the periods of active nitrous oxide production were in the range 1.8–28.1 $\mu g\ N_2O\text{-}N \cdot L^{-1} \cdot d^{-1}$. The data show the

TABLE 4. Denitrifying and nitrifying bacteria (ammonium oxidizers and nitrite oxidizers) in the Lake St. George water column. Den. = denitrifying bacteria; NH_4^+ = ammonium-oxidizing bacteria; NO_2^- = nitrite-oxidizing bacteria.

Depth (m)	Feb. 27, 1980			Jan. 21, 1981			Feb. 25, 1982	
	Den.	NH_4^+	NO_2^-	Den.	NH_4^+	NO_2^-	NH_4^+	NO_2^-
0				22	<1	1		
2				24	<1	2	1	1
3	170	1	1					
5	1700	1	1				1	1
8	790	1	1	35	<1	2	1	3
10	130	2	2					
12	79	1	1	11	<1	2		
14	240	1	1					
15				24	<1	2		

possibility for denitrification in the relatively anoxic water which develops during winter stratification. It is noteworthy that no nitrous oxide accumulated in the incubated samples in the absence of acetylene (data not shown), suggesting complete reduction to N_2.

Enumerations of bacteria at different depths in the water column in 1980, 1981, and 1982 showed denitrifiers in the range 11–1700 cells·mL^{-1} whereas nitrifier numbers were only of the order of 1 or 2 cells·mL^{-1} (Table 4). Acridine orange direct counts showed roughly 3×10^6 total bacteria·mL^{-1} (J. de Traversay and R. Knowles, unpubl. data).

Discussion

The data provide evidence of significant nitrification during January–March under the ice in Lake St. George. A similar phenomenon appears to have occurred in Wintergreen Lake (Wetzel 1983, p. 244). For Lake St. George, the average in situ rate of production of nitrite and nitrate was 13 μg N·L^{-1}·d^{-1} which was confirmed by bottle incubation experiments in the laboratory at in situ temperature. The occurrence of nitrapyrin- and acetylene-sensitive ^{14}C-bicarbonate incorporation (which according to Hall (1982) may underestimate nitrification) suggests that the nitrification was due to chemolithotrophic CO_2-utilizing nitrifiers (Billen 1976; Hynes and Knowles 1978) rather than heterotrophic organic carbon-utilizing nitrifiers (Hynes and Knowles 1982). However, only very low numbers of chemolithotrophic nitrifiers were detected. Less than 1 or 2·mL^{-1} were enumerated. Assuming a range of specific activities of 1.3–81 pg N·cell^{-1}·d^{-1} (Schmidt 1982), the observed in situ activity of 13 μg N·L^{-1}·d^{-1} would require a nitrifier population of from 1.6×10^2 to 1.0×10^4 cells·mL^{-1}. It appears, therefore, that the MPN enumeration provides an estimate which is 2 to 4 orders of magnitude below the true count, as has been reported by others (e.g. Ward et al. 1982), or other types of bacteria are responsible for the observed activity. Methanotrophic bacteria can also oxidize ammonia and are also inhibited by nitrapyrin (Topp and Knowles 1982) and acetylene (de Bont and Mulder 1976). It is unlikely that methanotrophs were significant contributors to the observed nitrification, since methane concentrations at all depths were below 0.5 μ*M* in 1980, 1982, and 1983, and only in 1984 did methane levels increase below the 8-m depth to 121 μ*M* in the bottom water (R. Knowles, unpubl. data). However, a methanotrophic population may have proliferated on the large amounts of methane made available during fall overturn (R. Knowles, unpubl. data).

It is unlikely that the observed increases in nitrite and nitrate could be due to inputs from outside the lake, since stream flow begins only at the end of March and then flows immediately under the ice (Wolfe 1979). Furthermore, any nitrogen oxide in groundwater could not survive passage through the rich sediments which have considerable denitrification potential (Knowles 1979). Indeed, no nitrate is observed in Lake St. George pore waters (R. Carignan, INRS-Eau, Quebec, unpubl. data).

The denitrification assays show that the water column has the potential to reduce nitrate to gaseous products whenever the oxygen is depleted below 0.3 mg O_2·L^{-1}. Our estimate of nitrification rates could be an underestimate if denitrification occurred in the sediment or water column during the periods studied.

The rates of oxygen consumption observed (mean of 75.6 μg O_2·L^{-1}·d^{-1}) are equivalent to 0.38 g O_2·m^{-2}·d^{-1} and as such are at the upper end of the range observed by Welch et al. (1976) for 16 Ontario lakes in winter. The fact that our observed nitrification must be associated with an oxygen consumption which is a significant percentage of the total observed oxygen depletion indicates that the nitrification process could frequently be a major factor contributing to winter-kill of fish. It is also very important in the nitrogen cycle relative to other processes. For example, the winter uptake of nitrogen by phytoplankton calculated from ^{14}C-bicarbonate uptake data was less than 0.5 μg N·L^{-1}·d^{-1} (D. R. S. Lean, unpubl. data); the sedimentation based on sediment trap data was 0.3 μg N·L^{-1}·d^{-1} (M. Charlton, Canada Centre for Inland Waters, unpubl. data); and diffusion of ammonium from the sediment, calculated from pore water concentration profiles and extrapolated to the whole lake, was 2 μg N·L^{-1}·d^{-1} (R. Carignan, INRS-Eau, Quebec, unpubl. data). By comparison, a nitrogen oxide production of 13 μg N·L^{-1}·d^{-1} is very significant.

Acknowledgements

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Measurements of Specific Rates of Net Methyl Mercury Production in the Water Column and Surface Sediments of Acidified and Circumneutral Lakes

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Specific rates of mercury methylation and demethylation were determined for water and surficial sediment samples taken from several lakes located in the Experimental Lakes Area, northwestern Ontario. Specific rates of mercury methylation were found to increase with decreasing pH in epilimnetic water samples in which pH was adjusted prior to incubation and in epilimnetic water samples taken from lakes of different pH. Reduction of pH also increased methyl mercury production at the sediment surface. Both increases and decreases in pH reduced specific rates of mercury demethylation. However, these changes were smaller than for methylation. Proportionally, specific rates of methylation increased faster than increasing concentrations of Hg^{2+}, while specific rates of mercury demethylation increased linearly with increasing concentrations of methyl mercury. Overall, this study predicts that the net rate of methyl mercury production in the water column and at the sediment–water surface will increase as a result of lake acidification, and this may at least partially explain why the mercury concentration of fish appears to increase during lake acidification.

Les auteurs ont déterminé les taux spécifiques de méthylation et de déméthylation du mercure dans des échantillons d'eau et de sédiments superficiels prélevés dans plusieurs lacs de la zone des lacs expérimentaux, au nord-ouest de l'Ontario. Ils ont découvert que les taux spécifiques de méthylation de mercure augmentent en fonction d'une baisse du pH des échantillons d'eau prélevés dans l'épilimnion quand le pH a été étalonné avant l'incubation, et des échantillons d'eau recueillis de l'épilimnion de lacs de différents pH. Une baisse du pH a aussi entraîné une augmentation de la production de mercure méthylé à la surface du sédiment. Les taux spécifiques de déméthylation du mercure ont été réduits par des augmentations et des diminutions du pH. Toutefois, ces variations étaient moins importantes que dans le cas de la méthylation. En proportion, les taux spécifiques de méthylation ont augmenté plus rapidement que l'accroissement des concentrations de Hg^{+2} tandis que les taux spécifiques de déméthylation de mercure ont augmenté de façon linéaire par rapport à l'accroissement des concentrations de mercure méthylé. En général, la présente étude prédit que le taux net de production de mercure méthylé dans la colonne d'eau et à l'interface sédiment–eau augmentera suite à l'acidification du milieu lacustre et ceci peut en partie expliquer pourquoi la concentration de mercure chez le poisson semble augmenter au cours de l'acidification de lacs.

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High mercury concentrations in fish harvested from acidified waters have been reported from the United States, Norway, and Sweden and have been the subject of a number of investigations (Jernelov et al. 1976; Scheider et al. 1979; Kelso et al. 1982; Wiener et al. 1983; R. J. P. Brouzes, Domtar Research Centre, Senneville, Que., pers. comm.). The main source of acidification and of many airborne trace metals, including mercury, is believed to be fossil fuel combustion which emits oxides of sulfur and nitrogen and metallic mercury. In the atmosphere these compounds are further oxidized to sulfuric acid, nitric acid, and mercuric ions and then enter the biosphere via precipitation (National Research Council 1981; Joensuu 1971).

This elevated mercury input to lakes might be an explanation for the increase in mercury noted in fish taken from acid-stressed, poorly buffered, inland waters. Acidification might also increase the solubility of mercury presently in lakes. Although Schindler et al. (1980) and Jackson et al. (1980) found that acidification of a softwater lake did not cause appreciable displacement of Hg^{2+} from bottom sediments, Miller and Akagi (1979) found that Ch_3Hg^+ was released from sediments to a greater extent at reduced pH.

In addition to geochemical considerations, the role of

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TABLE 1. Characteristics of 11 lakes in ELA showing change in pH in some lakes following experimental whole-lake acidification.

Lake	Lake surface area (ha)[a]	Maximum depth (m)[a]	Mean pH	Pretreatment pH[b]	Degree of eutrophication[b]
114	12.1	5.0	5.6	6.2	Mesotrophic
223	30.2	11.2	5.3	6.7	Oligotrophic
227	5.0	10.0	8.5	—	Eutrophic
239	56.1	30.4	7.0	—	Oligotrophic
302N	12.8	13.8	6.5	6.7	Oligotrophic
302S	10.9	10.6	5.5	6.6	Oligotrophic
303	9.9	2.5	8.0	—	Mesotrophic
373	28.3	>16.4	7.0	—	Oligotrophic
382	37.3	11.9	7.0	—	Oligotrophic
623	>30.0	>16.4	7.0	—	Oligotrophic

[a]Cleugh and Hauser (1971) and Brunskill and Schindler (1971).
[b]D. W. Schindler (pers. comm.).

aquatic microflora in the methylation of mercury and the possible enhancement of this activity at a reduced pH must also be considered. Hitherto, many researchers have considered the sediments as the most likely site for aquatic bacterial methyl mercury production although other sites such as the water column and fish intestinal contents have been identified (Furutani and Rudd 1980; Rudd et al. 1980). Accordingly, most previous microbiological investigations to account for the high mercury concentrations in fish from acidified lakes have centered on methylating activities in sediments to determine if lowered pH favors the formation of methyl mercury. For example, Ramlal et al. (1985) found that methylation rates decreased in sediment samples incubated anaerobically as the pH was lowered, and Baker et al. (1983) found that formation of methyl mercury in sediment samples only occurred at a pH range of 5.5–6.5. Considering these observations, it may seem contradictory that fish tend to have increased levels of mercury in lakes that have been stressed by acidic precipitation.

Enhanced production of methyl mercury in the water column of acidified lakes would have a direct influence on mercury distribution through the food chain and might account for higher mercury levels in fish tissue. To date, there is no information on the effect of acidification on microbial methylation in the water column. Since concentrations of methyl mercury, whether in the water column or at the sediment surface, represent the net difference between methylation and demethylation, and since lowered pH may well alter this methylation–demethylation balance, it seemed prudent to examine these relationships in the water column. In addition, methylation rates at the sediment–water interface under oxygenated lake water were examined to determine the effect of pH change.

Materials and Methods

Sampling Locale

The field work was conducted in the Experimental Lakes Area (ELA) northwestern Ontario, during the summer of 1984. Eleven lakes were sampled for this study; the area, maximum depth, trophic state, and average epilimnetic pH of the lakes are given in Table 1.

Lakes 114, 223, and 302S have received H_2SO_4 additions since 1979, 1976, and 1982, respectively, as part of an experimental acidification program. L302N is separated from L302S by a sea curtain and has received HNO_3 since 1982 as part of the same program. The initial pH values of L114, L223, L302N, and L302S were 6.0, 6.7, 6.7, and 6.6, respectively (Cruikshank 1984; Schindler and Turner 1982).

Measurement of Specific Rates of Mercury Methylation and Demethylation in Water

Samples of epilimnetic water from each lake were taken approximately 10 cm below the surface by submerging an 8-L polyethylene bottle. The samples were all well oxygenated. The bottle was rinsed twice with lake water prior to sampling. In some cases, methylation and demethylation activities were assayed without changing the ambient pH of the samples. In other cases, 1.2 L of a sample was transferred to a 2-L Erlenmeyer flask and H_2SO_4 or NaOH was added to appropriately adjust the pH. In either case, 150-mL subsamples were transferred to 300-mL BOD bottles for analysis of specific rates of mercury methylation and demethylation.

Specific rates of mercury methylation and demethylation were assayed by the radiochemical methods of Furutani and Rudd (1980) and Ramlal et al. (1986). The addition of $^{203}Hg^{2+}$ and $^{14}CH_3Hg^+$ to the samples increases the concentrations of available substrate well above natural concentrations. Natural mercury concentrations in these unpolluted ELA lakes are low (0.35 $\mu g \cdot g$ sediment^{-1} (Ramlal et al. 1985) and in water <5 $ng \cdot L^{-1}$ (R. Hunt, Freshwater Institute, pers. comm.)). Because of mercury or methyl mercury additions to the samples, neither of these methods assays absolute (natural) rates of activity. Rather, these methods yield specific rates of mercury methylation and demethylation, enabling relative comparisons of the effects that a particular environmental parameter, such as pH, has on rates of methylation and demethylation. When these two assays are used simultaneously, the effect of pH on the net rates of methyl mercury production can be determined by comparing the methylation to demethylation ratio of test samples (M/D) with the M/D of control samples (Ramlal et al. 1986). The main advantage of the radiochemical methods is their high sensitivity which allows measurements of both processes in the water column and sediments before natural microbial assemblages change.

For all methylation and demethylation experiments, each test consisted of three incubation bottles (two duplicate tests and an acid-killed control). We added 3.7×10^{-5} GBq of

$^{203}HgCl_2$ (3.7×10^{-5} GBq·μg Hg^{-1}) or 4.7×10^{-7} GBq of $^{14}CH_3HgI$ (1.68×10^{-6} GBq·μg Hg^{-1}) to each of the methylation or demethylation bottles. Prior to July 18, the samples were incubated at 22 ± 2°C in the dark for 24 h. Subsequently, it was found that the high sensitivities of the methods allowed us to reduce incubation times to 6 h. For certain time course experiments, incubation periods were extended up to 6 d. After incubation, methylating and demethylating activity was terminated by the addition of 1 mL of 4 *N* HCl or 3 *M* H_2SO_4.

Alkylated ^{203}Hg produced during the incubation period was extracted from the samples and counted by scintillation spectrometry as described by Furutani and Rudd (1980). The volatile ^{14}C produced by the demethylation process was trapped and assayed as described by Ramlal et al. (1986) except that a dimethylmercury trap of 100 g of KBr and 15 g of HgBr in 1 L of distilled water (Spangler et al. 1973) was put into the ^{14}C trapping apparatus.

Calculation of Specific Methylation and Demethylation Rates in Water

To examine the effect of mercury concentration on methylation rate, the methylation rate was calculated as follows:

$$Rm = (s - b)(d)(a^{-1})(t^{-1})$$

where Rm = rate of methylation (nanograms of mercury per litre per day), s = sample activity (dpm per litre of lake water), b = control activity (dpm per litre of lake water), d = dilution factor from extraction procedure, a = dpm added per nanogram of mercury added, and t = incubation time (days).

When the quantity of ^{203}Hg added to a sample was constant, Rm was expressed as a percentage of the mercury methylated as suggested by Ramlal et al. (1986):

$$Rm = \frac{100(s - b)}{C}$$

where C = quantity of isotope added to the sample (dpm per litre).

When the concentration of Ch_3Hg^+ added to water samples was varied, the specific rate of demethylation (nanograms of mercury per litre per day) was as follows:

$$Rd = (s - b)(e)(t^{-1})$$

where Rd = rate of demethylation (nanograms of mercury per litre per day) and e = dpm added per nanogram of Ch_3Hg^+.

When the amount of Ch_3Hg^+ added to a sample was constant, specific demethylation rates were calculated as percentages:

$$Rd = \frac{100(s - b)}{C}.$$

The M/D was calculated as follows:

$$M/D = \frac{Rm}{Rd}.$$

On several occasions, time course experiments of mercury methylation and demethylation were conducted using epilimnetic water samples (e.g. Fig. 1). In general, methylation and demethylation rates were constant for the first 12–24 h. Thereafter, methylation rates declined as rates of demethylation increased. Demethylation rates usually peaked after 5–6 d, while methylation rates slowed progressively after the first 12–24 h. Because of these results, incubation of samples was reduced from 24 to 6 h.

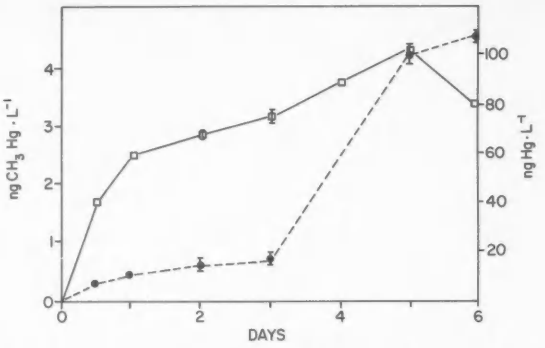

FIG. 1. Time courses of specific methylation (□) and demethylation (●) rates for L239 epilimnetic water (pH 7.0) sampled July 18, 1984. Means of duplicate samples are plotted. Vertical lines indicate the range of differences; very small ranges are not indicated.

Core Incubations

Mercury methylation at the sediment–water interface was investigated using intact sediment cores (5 cm I.D. × 20 cm) which were taken from epilimnetic sediment by a diver. These consisted of 8–11 cm of sediment with 9–12 cm of oxygenated overlying water and no air space. The pH of overlying water was adjusted by addition of H_2SO_4 and 7.4×10^{-5} GBq of $^{203}HgCl_2$ was then added to the overlying water. The cores were incubated at in situ temperatures.

After incubation for 1 d, the water phase (180–240 mL) was pipetted into a BOD bottle for $CH_3\ ^{203}Hg^+$ measurement (Furutani and Rudd 1980). Then the sediment was sliced into 1-cm sections, each of which was transferred into a BOD bottle with 100 mL of water for alkyl $^{203}Hg^+$ extraction.

Results

Rates as a Function of Hg or CH_3Hg Concentration

To evaluate the effect of mercury concentration on methylation, increasing concentrations of radiolabelled Hg^{2+} were added to L302S water samples. As the concentration of Hg^{2+} added was increased to 66.6 μg·L^{-1}, the rate of methylation appeared to increase to a proportionally greater extent than the Hg^{2+} concentration (Fig. 2). Similarly, in another sample taken from the same lake in September 1984, increasing the amount of added mercury by a factor of 2.6 resulted in a 5.6-fold increase in the rate of mercury methylation.

That the methylating activity observed was likely of biological origin is demonstrated by our control experiments where methylation was completely inhibited either by autoclaving the sample or by the addition of concentrated HCl. Nonbiological (chemical) methylation is not inhibited or influenced by autoclaving where the methylating agents are fulvic or humic acids (Nagase et al. 1982).

The effect of varying concentrations of methyl mercury on demethylation in L302S water samples is shown in Fig. 3. For added concentrations ranging from 0.0 to 7.46 μg of Hg added as $CH_3Hg^+ \cdot L^{-1}$, a linear rate of increase in demethylation was

TABLE 2. Effect of pH on specific rates of methylation and demethylation and net methylation (M/D) for epilimnetic water samples.

Date	Lake	pH	Rm ($\%\cdot L^{-1}\cdot d^{-1} \times 10^2$)[a]	Rd ($\%\cdot L^{-1}\cdot d^{-1} \times 10$)[a]	$M/D \times 10^2$
July 10[b]	239	7.1[b]	4.19 (0.03)	6.05 (0.27)	6.93
		6.5	5.67 (0.00)	6.64 (0.12)	8.54
		5.5	9.00 (0.21)	4.00 (0.18)	12.1
July 18	239	7.1[b]	2.51 (0.05)	6.12 (0.15)	4.10
		6.5	2.60 (0.05)	6.10 (0.54)	4.26
		5.9	3.35 (0.06)	5.71 (0.93)	5.87
		5.5	3.51 (0.08)	4.35 (0.02)	8.07
		5.1	5.82 (0.09)	2.81 (0.59)	20.7
Aug. 8	623	7.1[b]	11.57 (0.08)	20.58 (0.11)	5.62
		6.4	14.94 (0.17)	18.54 (0.90)	8.06
		5.5	18.00 (0.05)	16.82 (0.75)	10.70
		4.6	22.25 (0.03)	10.18 (0.16)	22.10
Aug. 23	114	7.0	3.24 (0.20)	8.07 (0.05)	4.01
		6.1	12.74 (0.33)	14.02 (0.16)	9.09
		5.6[b]	13.73 (0.44)	14.35 (0.11)	9.57
Sept. 11	239	6.94[b]	4.59 (0.15)	13.09 (1.87)	0.35
		6.24	10.62 (0.03)	12.59 (1.64)	0.84
		4.46	11.19 (0.09)	2.13 (0.26)	5.25
Sept. 12	302S	8.45	2.73 (0.06)	2.01 (0.01)	1.36
		6.65	5.64 (0.09)	6.98 (1.5)	0.81
		6.14	7.11 (0.15)	9.01 (1.91)	0.79
		5.56[b]	11.16 (0.33)	9.36 (1.82)	1.19

[a]Mean rate of two samples with range in parentheses.
[b]Untreated samples, initial epilimnetic pH.

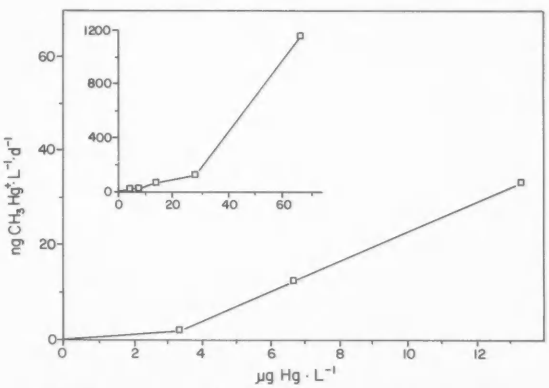

FIG. 2. Effect of added Hg^{2+} on specific rates of mercury methylation in L302S epilimnetic water sampled September 13, 1984. Inset is the same experiment over a wider concentration range of added Hg^{2+}

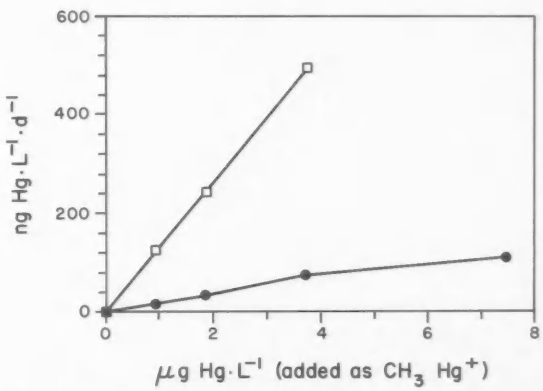

FIG. 3. Effect of added CH_3Hg^+ on specific rates of mercury demethylation in L302S epilimnetic water sampled September 13, 1984 (□), and September 18, 1984 (●).

observed up to 3.73 $\mu g \cdot L^{-1}$ above which the rate of increase appeared to decline.

Rates as a Function of pH

Specific mercury methylation and demethylation rates as a function of pH were examined. Since the net balance of methyl mercury in the ecosystem as a consequence of pH changes was of particular interest, M/D values were also determined. When pH of epilimnetic water samples from circumneutral L239 and L623 was lowered by additions of H_2SO_4, the methylation rates increased and demethylation rates decreased over the same pH range. The M/D, therefore, increased as the pH was lowered (Table 2). When the pH of epilimnetic water samples from acidified L114 was raised, specific methylation and M/D decreased. Similar results were found in L239 and L302S during September. Thus, in all but one instance, the

TABLE 3. Comparison of specific rates of methylation and demethylation and net methylation (M/D) in water samples from acidified L114, L223, L302N, and L302S, from circumneutral L623, L373, L382, from eutrophic L227, and from reference L239 and L303 during 1984.

Date	Lake	pH	Rm ($\%\cdot L^{-1}\cdot d^{-1} \times 10^2$)[a]	Rd ($\%\cdot L^{-1}\cdot d^{-1}$)[a]	$M/D \times 10^2$
July 8	303	6.6	2.24 (0.09)	0.57 (0.02)	3.93
	114	5.9	3.47 (0.06)	0.68 (0.15)	5.12
July 9	227	8.3	0.92 (0.00)	0.67 (0.07)	1.37
	239	7.1	4.08 (0.03)	0.61 (0.03)	6.74
July 23	239	7.1	5.16 (0.15)	0.70 (0.08)	7.39
	223	5.3	7.22 (0.75)	0.72 (0.06)	10.0
	302N	6.5	9.50 (0.05)	1.02 (0.14)	9.31
	302S	5.5	12.83 (0.65)	0.89 (0.06)	14.40
Aug. 8	623	7.1	8.31 (0.08)	2.06 (0.01)	3.93
	373	7.1	6.57 (0.03)	0.93 (0.09)	7.03
Aug. 16	239	7.0	7.00 (0.06)	2.28 (0.07)	3.06
	223	5.4	13.35 (0.08)	2.17 (0.01)	6.15
	302N	6.5	14.28 (0.11)	2.54 (0.43)	5.61
	302S	5.5	31.59 (0.90)	2.38 (0.09)	13.30
Aug. 17	303	8.5	4.79 (0.02)	2.31 (0.13)	2.05
	114	5.5	27.65 (0.24)	2.37 (0.06)	11.64
Aug. 19	382	6.8	8.00 (0.05)	1.62 (0.03)	4.95

[a]Mean rate of two samples with range in parentheses.

M/D increased with decreasing pH, indicating an increase in the rate of net mercury methylation.

The effect of long-term acidification of lakes on rates of methylation and demethylation in the water column was also examined. Unstratified L303 and L114, similar with respect to size and trophic status, were studied. Since 1979, L114 has received additions of H_2SO_4, and at the time of sampling, surface water pH was 5.5; L303 has been untreated and served as a reference "control" for L114. Water samples taken from L114 on July 8 and August 17 had higher specific methylation rates than those of L303 (Table 3) whereas specific demethylation rates in L114 and L303 were quite similar. The M/D of L114 epilimnetic water was much higher than for L303, particularly in August when the latter had a pH of 8.5, the result of an algal bloom.

Table 3 also presents data from three other experimentally acidified lakes, L223, L302N, and L302S, with unacidified L239 as a control. All three lakes are stratified and are of similar productivity (Table 1). Water samples from L302S yielded the highest specific methylation rate. Although L223 had the lowest epilimnion pH (5.3), its specific methylation rate was only moderately higher than that of reference L239. Demethylation activities were similar in water samples from all four lakes. Again, the M/D was higher at lower pH's.

Specific methylation rates in epilimnetic water samples from nonacidified lakes (L227, L623, L373, and L382) during July and August are also noted in Table 3. Of these, eutrophic L227 had the lowest specific methylation rate correlated with the highest pH (8.3) at the time of sampling. The other three lakes were oligotrophic, varied little in epilimnion pH, and displayed modest variations in methylating activity. Specific demethylation rates in these lakes were higher than that in L227, especially in the case of L623. The lowest M/D values observed during this study (1.37 and 2.05) were found in L227 and L303. At the time of the sampling, the pH of both of these lakes exceeded 8.0.

TABLE 4. Effect of pH on methyl mercury production in intact sediment cores sampled at a depth of 1 m in L239 on August 22 and 29, 1984.

Date	Initial water pH	Total $CH_3{}^{203}Hg^+$ in water (ng)	Total $CH_3{}^{203}Hg^+$ in sediment (ng)
Aug. 22	6.3[a]	2.91	0.79
	3.4	6.24	1.74
Aug. 29	6.3[a]	1.16	2.53
	6.3[a]	1.35	2.20
	6.3[a]	1.53	2.94
	5.4	2.99	2.71
	4.7	3.29	2.01
	3.4	4.99	6.39

[a]Untreated samples.

The sediment–water interface is likely to be an active site for mercury methylation in lake ecosystems (Robinson and Tuovinen 1984). Table 4 shows the marked increase in $CH_3{}^{203}Hg^+$ concentration, correlated with a decrease in pH, in the oxygenated overlying water of sediment cores. At a surface water pH of 3.4, there was also an increase in $CH_3{}^{203}Hg^+$ concentration in the surface sediments.

Discussion

Although mercury methylation and, to a lesser extent, demethylation in freshwater sediments have been studied by a number of investigators, reports of mercury methylation in the water column have been scarce (Furutani and Rudd 1980; Topping and Davies 1981). Prior to this report, we were not aware of any other publication describing demethylating activity in the water column. In our investigations, methylation and demethylation were consistently found in the water columns of a number of ELA lakes during the summer of 1984.

This is in contrast with the report by Furutani and Rudd (1980), who detected methylating activity in 5 of 13 trials in the water column of a mercury-polluted lake. In their case, the methylating activity appeared to be cyclical as noted by others working in other types of environments (Spangler et al. 1973; Edwards and McBride 1975; Hamdy and Noyes 1975).

Previous studies of abiotic and biotic methylation in sediments and in the laboratory have shown that methylation rates increase linearly with an increase in Hg^{2+} concentration (DeSimone et al. 1973; Nagase et al. 1982; Rudd et al. 1983). However, our study with water samples showed that the increase in methylation rate was proportionally greater than the increase in Hg^{2+} (e.g. Fig. 2). This may have been because in water, the proportion of unbound (microbiologically available) mercury increases at higher concentrations when available binding sites are saturated. This possibility warrants further investigation. We also found that demethylating activity was related to CH_3Hg^+ concentration, but in this case the rate of increase was linear with respect to increasing methyl mercury concentration (Fig. 3). Our finding that increasing substrate concentration stimulates methylation and demethylation in water in a different manner suggests that net methyl mercury production will increase in water as mercury concentrations rise.

We used two approaches to examine the effect of pH on the methylation and demethylation of mercury in the water column. First, we increased or decreased the pH of epilimnetic water samples and then compared the effects of these short-term changes on specific rates of methylation and demethylation. Second, we looked at long-term effects of pH by comparing specific methylation and demethylation rates in epilimnetic water samples from several lakes where the epilimnetic pH had been lowered by experimental acidification, raised by eutrophication, or was at a "natural" level. Both approaches consistently demonstrated that lowering the pH increased the specific methylation rate (Tables 2 and 3). For example, eutrophic L227, which had the lowest recorded specific methylation rate, had a very high pH. The short-term bottle incubations demonstrated that raising the pH of L302S water to 8.45 also resulted in low methylating activity (Table 3). Similarly, reduction in pH below the natural values increased the specific rates of methylation in short- or long-term experiments. These findings concur with those of Rudd and Turner (1983), who found that fish accumulated less mercury in an enclosure where the pH had been raised by stimulating primary production than in an enclosure where the water pH was unaltered.

It is well known that decomposable organic matter stimulates bacterial activity and mercury methylation under certain circumstances (e.g. Furutani and Rudd 1980). Thus, it might be expected that methyl mercury production would be faster in eutrophic lakes where decomposition rates are faster. However, the study of Rudd and Turner (1983) and this study suggest that in fact, methylation may be slower in eutrophic situations, as it was in L227 and in L303 during an algal bloom (Table 3). The reason for this appears to be that eutrophic lakes often have a high pH because of their high primary productivity. Our data suggest that this high pH is a very important controlling factor that is more important than the stimulatory effect of a fast decomposition rate.

An explanation for the increase in observed methylation rates at reduced pH could involve a number of interacting factors. For example, at low pH, because of an exchange of Hg^{2+} with H^+, there could be proportionately more free Hg^{2+} which can also cross the cell membrane more effectively at low pH (Bienvenue et al. 1984). This would enhance methylation by either the cobalamin-dependent (Wood 1980) or the homocysteine/methionine processes (Landner 1971) because methylation rate is dependent on the mercury concentration. An additional explanation might be that of Wood (1980), who predicted, on thermodynamic grounds, that methylation of Hg^{2+} by methylcobalamin should be enhanced at reduced pH and at positive redox potentials, both of which occur in the water column and at the sediment surface. DeSimone et al. (1973) also showed that methylcobalamin-dependent methylation has an optimum at pH 4.7.

Specific rates of demethylation decreased whether pH was raised or lowered from the ambient pH values (Tables 2 and 3). Any experimentally induced shift in pH may supress the enzymatic activities of the demethylating populations or interfere with cross-membrane transport of methyl mercury. Overall, however, for both the short-term bottle experiments and the long-term lake experiments, change in pH had a smaller effect on specific rates of demethylation than on methylation rates.

Because changes in pH affected methylation to a greater degree than demethylation, the consistent increase in the net rate of mercury methylation (M/D) that we routinely observed was primarily due to increased methylation at low pH. This relationship is clearly shown in Fig. 4. Net methyl mercury production in lake water was about seven times faster ($r = -0.85$) at low pH (approximately 4.5) than at high pH (approximately 8.5). Methyl mercury rapidly produced in the water column of acidified lakes is directly available for uptake by fish via the gill membranes.

While net methylation in the water column and at the sediment–water interface increased with decreasing pH, in anoxic sediments just below the sediment–water interface, net methylation decreased with decreasing pH (Ramlal et al. 1985; Furutani et al. 1984). This decrease of net methylation in the anoxic subsurface sediment is not due to a decrease in microbial activity at low pH (Furutani et al. 1984), but might be due to the binding of Hg^{2+} to free sulfides at reduced pH (Ramlal et al. 1985). A major difference between the previous sediment studies (Ramlal et al. 1985; Furutani et al. 1984) and this one is that these incubations were all of aerobic surface sediment, while the previous studies used anaerobic subsurface sediment. Wood (1980) predicted that a combination of low pH and high redox potentials would be optimal for mercury methylation. These conditions were found in the water column samples at low pH and at the sediment–water interfaces of the cores that we incubated (Table 4), but not in the anaerobically incubated subsurface sediments.

In order to relate these results to the actual lake environment, it should also be understood that in most acidified lakes, the porewater pH of subsurface sediments is maintained at near preacidification levels by anaerobic consumption of H^+ by bacteria (Kelly and Rudd 1984; Rudd et al. 1987) although this is not the case in a few very oligotrophic lakes (Rudd et al. 1987). Thus, unlike net methylation in the surface water and surface sediments, which appears to be stimulated at reduced pH in all lakes, the impact of surface water acidification on net rates of methylation in the subsurface sediment will likely vary from lake to lake according to the subsurface pH of the sediment porewater.

In summary, this study and previous studies (Furutani and Rudd 1980; Ramlal et al. 1985; Rudd et al. 1987) suggest that

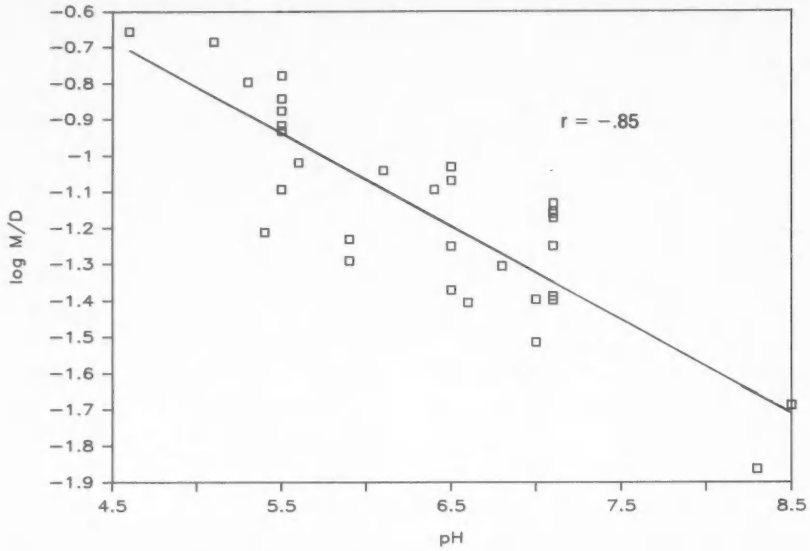

FIG. 4. Effect of pH on net rates of mercury methylation (M/D) for water samples taken from lakes of varying pH and for experiments in which the in situ pH of water samples was adjusted in the laboratory.

the whole-lake impact of acidification in most lakes will be an increase in net methyl mercury production in the water column and in the surface sediments, with methylation in the subsurface sediments being unaffected. In a few lakes where porewater pH is reduced, subsurface methylation will be reduced. In these cases, the whole-lake effect of acidification will be determined by the relative importance of net methylation in the water column and surface sediment versus the subsurface sediment. Our finding that reduced pH and elevated mercury concentrations can stimulate net methyl mercury production could be one explanation for the elevated concentration of mercury in fish in acidified lakes.

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Prey Choice among Piscivorous Juvenile Walleyes (*Stizostedion vitreum*)

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Lyons, J. 1987. Prey choice among piscivorous juvenile walleyes (*Stizostedion vitreum*). Can. J. Fish. Aquat. Sci. 44: 758–764.

In order to examine prey selection by walleyes (*Stizostedion vitreum*), I compared prey electivities for walleyes in Sparkling Lake, Wisconsin, with the results of species-preference experiments in the laboratory. Before young-of-the-year (YOY) yellow perch (*Perca flavescens*) were present in the littoral zone of Sparkling Lake, juvenile walleyes fed significantly less on minnows and more on darters than expected based on the relative abundance of these two taxa in the lake. After large numbers of YOY yellow perch appeared in the littoral zone, walleyes fed primarily on the abundant yellow perch, although their diet was not significantly different from that predicted based on the relative abundance of minnows, darters, and YOY yellow perch in the lake. In laboratory experiments, juvenile walleyes ate more bluntnose minnows (*Pimephales notatus*) than YOY yellow perch, and almost never ate johnny darters (*Etheostoma nigrum*). Walleyes were more successful at capturing bluntnose minnows than YOY yellow perch, but even when this was taken into account, walleyes ate more bluntnose minnows and fewer YOY yellow perch than expected, indicating that walleyes preferred bluntnose minnows to YOY yellow perch. Differences in prey selection between walleyes in the field and in the laboratory may have been related to differences among prey in distribution and movement patterns in Sparkling Lake.

Afin d'examiner le choix de proies chez le doré (*Stizostedion vitreum*), l'auteur a comparé le choix de proies du doré dans le lac Sparkling (Wisconsin) avec les résultats d'expériences de préférence selon l'espèce menées en laboratoire. Avant le peuplement de la zone littorale du lac Sparkling par la perchaude (*Perca flavescens*) de moins d'un an, les dorés juvéniles ont consommé nettement moins de ménés et plus de dards que prévu d'après l'abondance relative de ces deux taxons dans ce lac. Après l'apparition d'un grand nombre de jeunes perchaudes dans la zone littorale, le doré s'est nourri principalement de cette espèce, mais le régime n'était pas nettement différent de celui prédit d'après l'abondance de ménés, de dards et de jeunes perchaudes dans le lac. Au cours d'expériences en laboratoire, des dorés juvéniles ont consommé plus de ventres-pourris (*Pimephales notatus*) que de jeunes perchaudes et n'ont presque jamais consommé de raseux-de-terre (*Etheostoma nigrum*). Le doré a eu plus de succès dans la capture de ventres-pourris que de jeunes perchaudes mais même quand ce fait était considéré, il a consommé plus de ventres-pourris et moins de jeunes perchaudes que prévu, ce qui indique qu'il préfère la première espèce. Les différences pour ce qui est du choix de proies entre le doré en milieu sauvage et en laboratoire peuvent être liées aux différences de la distribution et des régimes migrateurs des proies dans le lac Sparkling.

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Young-of-the-year (YOY) yellow perch (*Perca flavescens*) are a major food item of juvenile walleyes (*Stizostedion vitreum*) in many lakes (Colby et al. 1979). Due to the timing of spawning, YOY yellow perch usually do not become available to walleyes in the littoral zone until early summer. When YOY yellow perch first appear there in large numbers, there may be substantial shifts in walleye diet to YOY perch and away from other prey (e.g. Swenson 1977). Usually it is unclear whether this switch occurs because YOY yellow perch are actively chosen by walleyes over other prey or because they are more abundant than other prey (Colby et al. 1979).

Although there is a large body of literature on the diet of walleyes (Colby et al. 1979), quantitative studies of prey electivity or choice by piscivorous walleyes are limited. In the few instances where they have been calculated, field estimates of prey electivity have been variable; in some lakes, YOY yellow perch appear to be actively selected (Forney 1974; Swenson 1977) whereas in others, they may be eaten in proportion to their abundance or even avoided in favor of other fishes (Wagner 1972; Knight et al. 1984). Laboratory studies on prey choice by walleye have generally been limited to YOY walleyes feeding on invertebrates or have concentrated on size rather than species selection (Mathias and Li 1982; Raisanen and Applegate 1983).

In 1983 the production of a large perch year-class in Sparkling Lake, Wisconsin, led to an increase in the impor-

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tance of YOY yellow perch in juvenile walleye diets. This change in consumption patterns provided an opportunity to examine species selection by piscivorous walleyes in the field, as well as a framework within which to investigate species preference in the laboratory. This paper uses both the field and laboratory results to address two specific questions: (1) Could the increase in the importance of YOY yellow perch in the diet of juvenile walleyes in Sparkling Lake be explained solely by the increase in YOY yellow perch abundance, or was there evidence that perch were eaten in preference to other prey? (2) How well could laboratory estimates of walleye prey preference explain or predict walleye diets in Sparkling Lake?

Materials and Methods

Field Studies

Walleyes in the size range 125–250 mm total length (TL) were captured during June and July 1983 from Sparkling Lake, a small (88 ha surface area, 11 m mean depth) mesotrophic lake in north-central Wisconsin. All were juveniles (age I+ or II+). Most were taken after dark with a boat-mounted AC electroshocker, although a few were caught in overnight sets of 25-mm-mesh fyke nets (Lyons 1984). All were measured to the nearest millimetre (TL), and their stomach contents were removed by gastric lavage (Light et al. 1983). Fishes found in stomachs were identified to the lowest taxonomic level possible and counted.

Prey population sizes were estimated from catch-per-effort data (Lyons 1987). Standardized seine hauls were made at 30 randomly chosen stations in the littoral zone of the lake. Using independently derived estimates of seine efficiency (Lyons 1986), seine catch per unit area for each prey species was converted to number of fish present per unit area. Densities of prey in water too deep to seine were estimated using the ratio of the small-mesh fyke net catch or SCUBA transect count in water too deep to seine to the catch or count in water shallow enough to seine. Densities were converted to numbers of fish based on the bottom area of each depth stratum in the lake. The numbers in all depth strata were then summed to give the total population size in the lake for each prey species.

Twenty-five fishes were captured in the seine, but because of insufficient data on seine efficiency and depth distribution, precise population estimates could only be made for bluntnose minnows (*Pimephales notatus*), *Notropis* spp. (primarily mimic shiners, *N. volucellus*) johnny darters (*Etheostoma nigrum*), Iowa darters (*E. exile*), and logperch (*Percina caprodes*) (Lyons 1978). YOY yellow perch population size could not be precisely estimated from catch-per-effort data, but based on walleye consumption estimates and changes in seine catch per effort, it was possible to estimate minimum and maximum population sizes during early July 1983 (Lyons 1984). Bioenergetics modeling indicated that walleyes consummed about 700000 YOY perch between July and October 1983. During this period, seine catch-per-effort of YOY perch declined about 75%. If all of this decline is assumed to be due to walleye consumption, then there had to be nearly 1 000 000 YOY perch present in early July. This is a minimum estimate because other predators also consumed YOY perch and because offshore movements may have caused part of the reduction in seine catch (Lyons 1984, 1987). However, at the least, walleye consumption was probably responsible for half of the decline in seine catch-per-effort of YOY perch; this yields an estimate of about 2 000 000 for the maximum population size of YOY perch present in early July (Lyons 1984).

Some forage fish were too large to be eaten by juvenile walleyes. The fractions of forage populations vulnerable to walleye predation were determined from equations relating walleye length to the maximum length (millimetres) of prey consumed (Knight et al. 1984):

$$\text{Maximum yellow perch length} = -215.785 + 55.941\,(\log_e \text{ walleye length})$$

$$\text{Maximum minnow or darter length} = -295.539 + 73.655\,(\log_e \text{ walleye length}).$$

The median length of all age I+ and II+ walleyes (combined) captured during each month was used to determine the maximum length of prey that could be consumed. The proportion of each prey population vulnerable to walleye predation was determined from seine catch data; for each prey species, up to 50 randomly chosen individuals per seine haul were measured to the nearest millimetre (TL). This proportion was multiplied by the total population size of that species to estimate the total number small enough to be vulnerable to walleye predation.

Diet and prey abundance data were grouped as minnows (bluntnose minnows and *Notropsis* spp.), darters (johnny and Iowa darters and logperch), or YOY yellow perch. For each sampling period, contingency tables and chi-square analysis (Snedecor and Cochran 1980, p. 125) were used to compare the number of vulnerable prey with both the number of walleye stomachs containing those prey and the total number of individuals of those prey observed in stomachs. For each prey, standardized chi-square residuals $[(\text{observed} - \text{expected})/(\text{expected}^{1/2})]$ were calculated as a measure of electivity (Newman and Waters 1984). These residuals represented the deviations between observed diets and abundance in the environment, with positive values indicating overrepresentation in the diet, negative numbers underrepresentation, and zero values an exact agreement between the relative amounts in the diet and in the environment (Newman and Waters 1984). Chi-square residuals were used rather than traditional electivity indices (e.g. Ivlev's) because there are difficulties in relating traditional indices directly to the results of statistical comparisons (e.g. Kohler and Ney 1982) and because chi-square analyses were also used to analyze laboratory results and thus test statistics for field and laboratory data were directly comparable.

Laboratory Studies

Prey preferences of juvenile walleyes from Sparkling Lake were studied in the laboratory from October to December 1983. During early October, three YOY walleyes were captured from Sparkling Lake with the electroshocker and transferred to three circular 500-L tanks at the Laboratory of Limnology, Madison, for use in prey choice experiments. Due to unusually fast growth during the preceding summer, these fish had lengths and weights similar to those of the larger I+ and smaller age II+ walleyes whose diets were examined in the field in June and July (Lyons 1984). The three YOY walleyes had probably fed primarily on YOY yellow perch since July (Lyons 1984). Once in Madison, they were held for 2 wk at 18–21°C and offered macroinvertebrates (crayfish, aquatic insects, and leeches) daily as food prior to the initiation of prey choice experiments.

Several procedures were followed in all experiments. Temperature was held between 16 and 23°C and did not vary more than 4° during a 24-h period. Three banks of fluorescent lights were on for 14 h each day. On and off times of each bank were staggered so that there were 1-h periods of changing light intensity from 17:00 to 18:00 and from 07:00 to 08:00 Central Standard Time. During dark periods, very low levels of illumination were provided by reflected streetlights from outside. At the start of each experiment, and each morning for the duration of each experiment, the walleyes were lightly anesthetized with MS222, weighed and measured (TL), had their stomachs emptied with gastric lavage, and were returned to their tanks. This did not suppress feeding by the walleyes, since each walleye always ate at least one fish per trial, and since laboratory consumption rates at high prey densities ($33-112\ mg \cdot g^{-1} \cdot d^{-1}$) overlapped with field estimates of maximum consumption rates ($30-40\ mg \cdot g^{-1} \cdot d^{-1}$; Swenson 1977). Daily gastric lavage had three advantages: (1) It standardized stomach volume (and presumably hunger levels) at the beginning of each trial. (2) It allowed examination of stomach contents. Through relative state of digestion it was possible to determine the order of prey consumption in multispecies trials. (3) It helped prevent substantial growth from occuring during experiments. The following changes in TL (millimetres) and wet weight (WW, grams) were observed over the entire experimental period (October 16–December 11):

Walleye	Initial/final TL	Initial/final WW
A	209/218	61/60
B	214/227	69/72
C	179/181	40/43

The first experiment examined the effects of the relative abundance of three prey species on walleye prey choice. During each trial, each walleye was exposed to three prey: bluntnose minnows, johnny darters, and YOY yellow perch. Each prey species was present at either high (six fish) or low (two fish) levels. Eight trials covered all possible combinations of high and low levels of the three prey. The order of trials was randomized for each walleye and new prey were used in each trial. To minimize size effects, in all experiments similar length ranges of each prey (45–65 mm TL) were used. Preliminary observations indicated that the three walleyes rarely fed during periods of full illumination. Therefore, for each trial, prey were added between 16:00 and 17:00 and walleye stomachs were examined between 08:00 and 09:00 the next morning.

The second experiment examined walleye prey choice between YOY perch and bluntnose minnows. In each of eight trials, each walleye was exposed to approximately 12 bluntnose minnows and 12 YOY perch. The actual number varied from 10 to 13 because of prey death during a trial due to unknown causes, and miscounts. Prey were added and walleye stomachs checked in the same manner as in the first experiment. Eaten fish were replaced with new fish for each trial, but uneaten fish were left in the tank.

The third experiment investigated the relationship between walleye feeding rate and prey density for bluntnose minnows and YOY yellow perch. Each walleye was exposed to 1, 2, 3, 5, 7, 9, or 10 individuals per trial of one prey type, and the number eaten was noted. Prey were added and walleye stomachs checked in the same manner as in the first experiment. The order of densities was randomized and new prey were used in each trial.

Data from the third experiment were fitted by nonlinear regression to the following model:

$$(1)\quad \text{Number of prey eaten} = \frac{a\ (\text{Number of prey present})}{[1 + a(h)\ (\text{Number of prey present})]}.$$

The parameters a and h are estimated regression coefficients that have ecological relevance: a is the attack coefficient, a measure of successful encounter rate, and h is handling time (Hassell 1978; Hewett 1980). Data from all three walleyes were combined to calculate these two parameters, but separate curves were fitted for the two prey types. For many species, a and h can be measured directly by observation (e.g. Werner 1977). Despite trying a wide variety of techniques, I was never able to observe walleyes in the act of feeding and had to estimate these parameters indirectly.

For a two-prey system, differences in attack coefficient (a) can be used to predict relative numbers of each prey eaten based on successful encounter rate by using the following formula (Hassell 1978):

$$(2)\quad \frac{\text{Relative no. eaten prey 1}}{\text{Relative no. eaten prey 2}} = \frac{a_1\ (\text{No. of prey 1 present})}{a_2\ (\text{No. of prey 2 present})}$$

where a_1 is the attack coefficient for prey 1 and a_2 is the attack coefficient for prey 2.

The a values for bluntnose minnows and YOY yellow perch from the third experiment were substituted in equation 2 to determine the expected number of each prey eaten by walleyes in the second experiment. Expected numbers were compared with actual numbers eaten by each walleye by pooling the results of chi-square analysis of each trial using the Mantel–Haenzel procedure (Mantel 1963). The results from all three walleyes were then also pooled using the same procedure. The number of times bluntnose minnows and YOY yellow perch were eaten first in the second experiment were also compared with expected values, calculated from equation 2, using chi-square analysis (Snedecor and Cochran 1980).

Results

Field

In early June 1983, when YOY yellow perch were absent from the littoral zone of Sparkling Lake, the main fishes in the diet of juvenile walleyes were minnows and darters. Although by weight minnows and darters dominated walleye diet, they were found in only 18% of the stomachs examined. Walleyes ate similar numbers of minnows and darters, even though vulnerable-sized minnows were four times more abundant (Table 1). No stomach contained both types of prey. Consumption patterns were significantly different from random based on both number of stomachs containing each prey and total numbers of each prey in stomachs (Table 2). Thus, based on their abundance in the environment, darters were overrepresented and minnows were underrepresented in the diets of juvenile walleyes during June.

In late June, YOY perch appeared in the littoral zone in large numbers. These perch had well-developed scales and spines. They were at least 24 times more abundant than

TABLE 1. Number of vulnerable-sized prey and diet of juvenile walleyes in the littoral zone of Sparkling Lake during June and July 1983.

Prey	Number of vulnerable prey (thousands)	Number of stomachs containing prey	Total number of prey in stomachs
June			
Minnows	40.6	6	7
Darters	8.6	8	9
YOY perch	0	0	0
		(79 examined)	
July			
Minnows	35.7	2	2
Darters	6.0	0	0
YOY perch	1000–2000	42	459
		(50 examined)	

TABLE 2. Standardized chi-square residuals and chi-square values for comparisons of juvenile walleye diets with abundance of vulnerable prey in Sparkling Lake during 1983. The numbers in parentheses refer to the numbers of YOY yellow perch present. Two July calculations were made, one assuming 1 million YOY yellow perch and the other 2 million. M = minnows, D = darters, YOY = YOY yellow perch. (A) Number of stomachs containing prey vs. abundance; (B) total numbers of individuals in stomachs vs. abundance.

	Standardized residuals				
	M	D	YOY	χ^2	p
(A) Number of stomachs					
June (0)	−1.34	2.24	—	8.59	<0.005*
July (1 million)	0.41	−0.45	−0.03	0.40	0.75
July (2 million)	1.34	−0.32	−0.17	1.99	0.40
(B) Total no. in stomachs					
June (0)	−1.35	2.19	—	8.94	<0.005*
July (1 million)	−2.84	−1.34	0.54	14.62	<0.005*
July (2 million)	−1.89	−1.05	0.29	5.43	0.07

minnows and darters combined. By July, most walleyes had switched to eating YOY perch (Table 1). YOY perch dominated walleye diet by both weight and number and were present in 84% of the stomachs examined. No adult darters were found in walleye stomachs (one stomach contained a few YOY logperch along with many YOY yellow perch), and only two stomachs contained minnows. Neither of the two stomachs which contained minnows contained YOY perch. Based on the number of stomachs containing each prey, consumption patterns were not significantly different from those expected based on relative abundances of prey, regardless of whether the YOY perch population size was 1 million or 2 million fish (Table 2). When total number of each prey in stomachs was considered, the number of YOY perch present was important. If 1 million perch were assumed, walleye consumption of minnows and darters was less than expected, and consumption of YOY perch was greater than expected, based on the relative abundance of each prey. However, if 2 million perch were assumed, walleyes are prey in proportion to the numbers of vulnerable prey in the lake (Table 2).

Laboratory

The first experiment indicated that the relative abundance of prey had an influence on walleye diet, although johnny darters were almost never eaten regardless of their abundance. The numbers of bluntnose minnows and YOY perch eaten were both positively related to their respective abundances (Table 3).

TABLE 3. Prey choice of three juvenile walleyes (A, B, C) at eight different combinations of prey densities. For each walleye the order of the eight trials was randomly chosen. The numbers in parentheses under the column totals are the number of times that prey type was eaten first in a trial. M = bluntnose minnow, D = johnny darter, and YOY = YOY yellow perch.

Number available			Number eaten								
			Walleye A			Walleye B			Walleye C		
M	D	YOY	M	D	YOY	M	D	YOY	M	D	YOY
2	2	2	0	0	1	1	0	1	2	0	1
2	2	6	1	1	0	2	0	1	1	0	1
6	2	2	4	0	0	1	0	0	3	0	1
6	2	6	2	0	0	1	0	1	3	0	2
2	6	2	1	0	1	1	0	0	2	0	1
2	6	6	1	0	3	1	0	1	1	0	1
6	6	2	3	0	0	3	0	0	3	0	0
6	6	6	2	0	0	2	0	1	2	0	0
32	32	32	14	1	5	12	0	5	17	0	7
			(6)	(0)	(2)	(6)	(0)	(2)	(6)	(0)	(2)

In the first experiment, bluntnose minnows were more likely to be eaten than YOY perch at all combinations of bluntnose minnow and YOY perch abundances (Table 3). Bluntnose minnows were the first fish consumed in 75% of the trials. YOY perch were most likely to be eaten first in those trials in which they were more abundant than bluntnose minnows.

In the third experiment, walleyes ate bluntnose minnows at a higher rate than they ate YOY perch (Fig. 1). From equation 1, a was 1.97 for bluntnose minnows and 1.28 for YOY yellow perch, while h was 0.21 for bluntnose minnows and 0.25 for YOY yellow perch. Thus, the walleyes had a higher successful encounter rate and a shorter handling time for bluntnose minnows than for YOY perch.

When bluntnose minnows and YOY yellow perch were present in equal numbers, the ratio of attack coefficients for the two species indicated that walleyes should consume 1.6 times more bluntnose minnows than perch, even when equal numbers of each prey were attacked (equation 2). This ratio was used together with the relative abundance of each prey to derive expected values for the numbers of bluntnose minnows and YOY perch eaten in the second experiment. Walleye A ate prey in proportion to the amounts predicted, but walleyes B and C ate more bluntnose minnows and fewer YOY perch than expected (Table 4). Pooled results from all three walleyes, for both total numbers of each prey eaten and number of trials in which each prey was eaten first, also indicated a higher consumption of bluntnose minnows than expected based on successful encounter rates and relative abundance (Table 4).

Discussion

YOY yellow perch probably dominated the diet of juvenile walleyes in Sparkling Lake in July 1983 because they were far more abundant than other prey taxa rather than because they were actively selected by walleyes. Selection for YOY perch was indicated only when the total number of prey in stomachs was considered and the minimum population size of 1 million

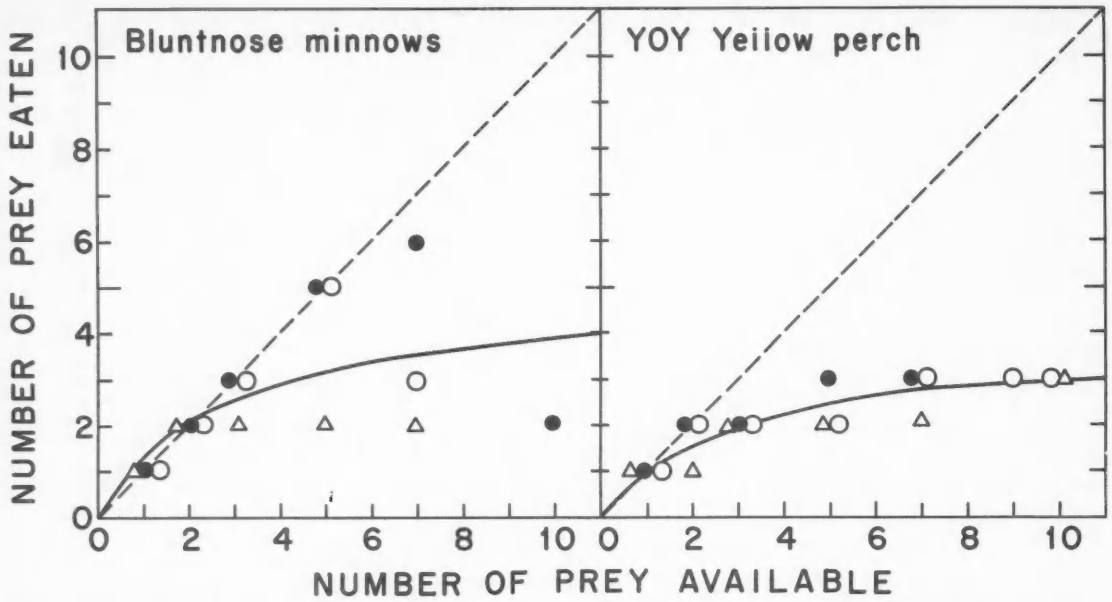

FIG. 1. Number of prey available vs. number of prey eaten for juvenile walleyes (Δ, walleye A; ●, walleye B; ○, walleye C) feeding on bluntnose minnows or YOY yellow perch in the laboratory. Only one prey type was available during each trial of approximately 15 h. The solid lines represent fits to the equation $y = ax/(1 + ahx)$ where y = number of prey eaten, x = number of prey available, a = successful attack coefficient, and h = handling time. For bluntnose minnows, $a = 1.97$ and $h = 0.21$; for YOY yellow perch, $a = 1.28$ and $h = 0.25$. The broken lines represent the maximum number of prey available.

TABLE 4. Pooled results of walleye prey choice tests for bluntnose minnows (M) and YOY yellow perch (YOY). For each walleye there were eight trials, in each of which approximately 12 bluntnose minnows and 12 YOY perch were present. Expected numbers eaten were calculated from equation 2 using attack coefficients (a) derived from single-prey trials (third experiment). Chi squares were calculated from pooled data using the Mantel–Haenszel procedure (Mantel 1963) to combine contingency tables from each trial. Numbers in parentheses refer to the number of trials in which bluntnose minnows or YOY perch were eaten first; expected values were calculated from equation 2.

	Total number available		Total number eaten		Expected Number eaten			
Walleye	M	YOY	M	YOY	M	YOY	χ^2	p
A	96	97	14 (7)	7 (1)	12.7	8.3	0.14	0.91
B	98	94	23 (8)	0 (0)	14.2	8.8	14.73	<0.005*
C	101	95	21 (7)	2 (1)	14.2	8.7	6.12	0.011*
Grand total	295	286	58	9	41.1	25.9	16.83	<0.005*
			(22)	(2)	(14.7)	(9.3)	6.17	0.012*

YOY perch was assumed. Laboratory results indicated that an increase in the relative abundance of YOY perch that was much smaller than that observed in Sparkling Lake could lead to a substantial increase in the proportion of YOY perch in the diet of juvenile walleyes.

Laboratory experiments also indicated that, when available in similar numbers, minnows were more likely to be eaten than darters or YOY perch, while YOY perch were more likely to be eaten than darters. In part, this could be explained by attack coefficients; walleyes were more successful at catching bluntnose minnows than YOY perch. However, even when attack coefficients were taken into account, walleyes still ate more bluntnose minnows than expected, indicating a preference. This preference was associated with a shorter handling time for bluntnose minnows, a response consistent with optimal foraging theory (Pyke et al. 1977) and earlier prey choice experiments with other piscivorous fishes (Werner 1977). The yellow perch's relatively deep body and sharp spines may have contributed to their slower handling time and lower successful attack coefficient. In other laboratory experiments, piscivores generally consumed relatively more cylindrical, soft-rayed species (i.e. bluntnose minnows) than deeper-bodied, spiny species (Lewis et al. 1961; Lewis and Helms 1963; Weithman and Anderson 1977; Moody et al. 1983).

There is some individual variability in walleye prey choice. The three prey taxa considered here were not found together in

walleye stomachs from Sparkling Lake, although during June this may have been a consequence of the relatively small numbers of stomachs examined that contained these taxa. In the second laboratory experiment the diet of walleye A could be explained solely on the basis of differential capture probabilities for bluntnose minnows and YOY perch, while walleyes B and C actively selected bluntnose minnows. Thus, variability in the overall diet of walleye populations might be due to individual walleyes concentrating on certain prey types, rather than each eating a wide variety of prey. Variability in diet might also result from differences in prey distribution relative to individual walleyes (see below).

Laboratory determinations of walleye prey choice could not have accurately predicted juvenile walleye diet in Sparkling Lake in 1983. In June, walleyes ate more darters and fewer minnows than would have been predicted, while in July they ate fewer minnows and more YOY perch. This may have occurred because prey population estimates did not accurately reflect the availability of prey to walleyes in Sparkling Lake. Prey availability in the field is not only a function of prey abundance, but also of distribution and behavior of both walleye and prey (Swenson 1977; Ryder and Kerr 1978). In the confines of a laboratory tank the influence of distribution and behavior on prey availability is probably reduced.

The distribution and behavior of walleye and their prey may have been important in determining prey availability in Sparkling Lake. During daylight hours in June and July of 1983, nearly all minnows in Sparkling Lake were found along the shoreline in water shallower than 2 m (Lyons 1987). Darters were common in this area as well but were present in moderate numbers out to the 5-m contour, and YOY yellow perch and juvenile walleyes were probably most common deeper than 2 m. Comparisons of day and night shoreline seine hauls during August 1981 (data from Long Term Ecological Research (LTER) — Northern Lakes of Wisconsin. Fish Database. National Science Foundation LTER Project: J. J. Magnuson, Center for Limnology, principal investigator) indicated that catches of YOY perch increased significantly at night (Mann–Whitney U test; $p < 0.05$), while darter and minnow catches did not change. This suggests that perch moved inshore at dusk and offshore at dawn, while the distribution of minnows and darters remained fairly constant. Many juvenile walleyes, which probably feed little, if at all, during the day in highly transparent lakes such as Sparkling (Ryder 1977), also moved inshore at dusk and offshore at dawn (personal observations). Thus, even though minnow and walleye distributions overlapped during much of the night, the distributions of walleye, YOY perch, and darters probably overlapped to a greater extent, particularly during twilight when diurnal species may be most vulnerable to crepuscular predators (Helfman 1981). Also, johnny darters are relatively active at night, while perch and minnows tend to rest quietly on the bottom (Emery 1973; Helfman 1979, 1981). Because of their movements, darters may have been more conspicuous to walleyes than minnows, and hence more likely to be attacked and eaten during June when YOY perch were not present.

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Genetic Relationships among Populations of Alaskan Chinook Salmon (*Oncorhynchus tshawytscha*)

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Chinook salmon (*Oncorhynchus tshawytscha*) collected from 13 Alaskan drainages were genetically characterized at 28 protein coding loci using starch–gel electrophoresis. Chinook salmon in western Alaska are generally quite similar to each other but are distinct from the more diverse southeastern Alaskan populations. Genetic compositions of southeastern Alaskan populations are generally intermediate between those of western Alaska and previously studied non-Alaskan populations to the south. Given that chinook salmon survived the Wisconsin glaciation in both the Bering and Pacific refuges, we propose that chinook salmon from both refuges participated in the post-Wisconsin colonization of southeastern Alaskan rivers.

À l'aide de l'électrophorèse sur gel d'amidon, les auteurs ont déterminé les caractéristiques génétiques de 28 locus codeurs de protéines chez des saumons quinnats (*Oncorhynchus tshawytscha*) capturés dans 13 bassins versants de l'Alaska. En général, les saumons quinnats de l'ouest de l'Alaska se ressemblent mais ils sont distincts des populations du sud-est de l'Alaska, plus diversifiées. Les compositions génétiques de ces dernières populations sont généralement intermédiaires entre celles des populations de l'ouest de l'Alaska et celles des populations d'autres régions du sud déjà étudiées. Étant donné que le saumon quinnat a survécu à la glaciation du Wisconsin dans les refuges de Béring et du Pacifique, les auteurs formulent l'hypothèse que le saumon quinnat de ces deux refuges a participé à la colonisation post-glaciaire des cours d'eau du sud-est de l'Alaska.

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Chinook salmon (*Oncorhynchus tshawytscha*) are distributed in western North America from the Ventura River in southern California to Point Hope in northeastern Alaska and in eastern Asia from northern Hokkaido to the Anadyr River (McPhail and Lindsey 1970). A variety of anadromous life cycles are found within the natural range of this species. Different populations return to their natal streams to spawn in the spring, summer, or fall. Juveniles may rear in freshwater for 1 or 2 mo (ocean-type) or a year or more (stream-type) before migrating to the sea (Healey 1983). Some populations spawn very close to salt water, while others migrate over 1000 mi up long rivers such as the Yukon to their spawning grounds. The variety of life histories, restricted potential for gene flow because of homing behavior, and the broad geographical distribution suggest that considerable genetic diversity may exist among populations of chinook salmon.

All Alaskan chinook populations that have been studied return in late spring or early summer to spawn and are stream-type fish (Healy 1983). However, because of the turbidity of many of the river systems and the remoteness of chinook spawning grounds, information about the biology and distribution of Alaskan chinook salmon is incomplete. At least one river system, the Kenai River draining into Cook Inlet, is characterized by two runs that spawn at different times (July and August) and in different areas within that drainage (Burger et al. 1985).

Information pertaining to diversity and number of populations of fish in a region can often be obtained from genetic compositions of collections of fish taken throughout the region. The genetic structures of fish populations may be used for stock identification by managers (e.g. Grant et al. 1980), for brood stock selection and management in hatcheries (e.g. Ryman and Ståhl 1980), and for inferring relationships among populations (e.g. Utter et al. 1980). Starch–gel electrophoresis has proven fruitful for examining the genetic compositions of chinook salmon in the Columbia River and western Washington (e.g. Milner et al. 1981, 1983). Characterization of the genetic compositions of these chinook populations has revealed differences among populations related to their watershed and to the timing of spawning runs. Studies in British Columbia indicate allozyme differences related to juvenile life history (Carl and Healey 1984). To obtain more information on the distribution and diversity of Alaskan chinook salmon populations and data that may be useful to managers and culturists, we have genetically characterized populations covering a broad geographical range of Alaska. In this paper, we examine genetic relationships among Alaskan chinook populations and compare them with some more southerly non-Alaskan, wild populations previously described by Milner et al. (1983).

Materials and Methods

Samples

Thirty-seven collections of young chinook salmon or tissue samples from adults, representing more than 2500 individuals,

FIG. 1. Sites of collection of Alaskan chinook salmon from western (left) and southeastern Alaskan (right) drainages. Numbers correspond to collections listed in Table 2. Collections from the same drainage which were pooled for analysis are designated by letters A–O and correspond to those in Tables 2, 3, and 4.

were taken from or near 13 major Alaskan river systems (Fig. 1; Table 2). Juveniles and smolts were captured with baited minnow traps and seines. Tissue samples of eye, liver, heart, and skeletal muscle were taken from adult fish netted in test fisheries, fish sacrificed in remote gamete collections for hatchery brood stock, and from commercial plants processing chinook caught by gill net. Samples were kept on ice until they were frozen and stored at −20 or −80°C. In most river systems, collections were made at more than one location and/or in more than one year.

Western Alaskan drainages sampled were the Unalakleet, Yukon, and Kuskokwim rivers and Bristol Bay. Chinook caught in Bristol Bay gillnet fisheries (see C, Fig. 1; Table 2) off the Igushik and Togiak rivers were sampled at processing plants in Dillingham and Togiak, respectively. Fish from gillnet fisheries in the lower Kuskokwim River (D) were sampled at plants in Bethel. Chinook caught in the lower Yukon (F and G) were sampled at processors and from a test fishery near Emmonak. These collections were made at different times during the run and in different years. Other collections from the Yukon drainage were taken from tributaries to the Tanana River system (H) in interior Alaska. From the northern-most river system, the Unalakleet River (E), collections of tissues were obtained from test fisheries.

In south-central Alaska, juveniles were collected from the Indian River, a Susitna River tributary (B). Adult chinook intercepted by the gillnet fishery as they returned to Copper River (A) were sampled at processing plants in Cordova.

In southeastern Alaska, six river systems were sampled, the Taku, Stikine, Chilkat, Farragut, Unuk, and King Salmon (Admiralty Island) rivers. Samples of tissues from adults were taken during remote gamete collections from King Salmon (K) and Farragut (N) rivers, Andrew Creek (M), a Stikine River tributary, and from the Chilkat drainage (I). Juveniles were also sampled from the Chilkat system (I), the Stikine River and upper tributaries (L), the Unuk River (O), and both the lower Taku River system and upper Taku River tributaries (J). The Taku and Stikine rivers are transmountain systems, crossing the coastal range into Canada near the headwaters of the Yukon.

Electrophoresis

Sample preparation and electrophoresis followed Utter et al. (1974). Small juveniles were macerated in water; larger juveniles were dissected to obtain individual tissues. Buffers used for electrophoresis were I (Ridgway et al. 1970), II (Markert and Faulhaber 1965), III (Clayton and Tretiak 1972), and IV, a pH 7.0 tris(hydroxymethyl)aminomethane buffer described by Shaw and Prasad (1970). The enzymatic activities were stained using methods described by Harris and Hopkinson (1976) and Shaw and Prasad (1970) modified for use in our laboratory (McGregor 1982; Lane 1984). Genetic loci and related data are given in Table 1. *Pep(Lgg)* is peptidase B activity. *Pep(Pp-2)* is peptidase D activity (Frick 1983).

Analysis

Departure of the genotypic frequencies of collections from

TABLE 1. Protein coding loci, their Enzyme Commission (EC) numbers (International Union of Biochemistry 1984) and designations (May 1980), and the tissues and buffers in which they were resolved. The peptidase loci are designated according to their substrate specificity. Buffers used are numbered as in the text.

Enzyme	EC No.	Designation	Tissue	Buffer
Alcohol dehydrogenase	1.1.1.1	*Adh*	Liver	II, III
Aspartate aminotransferase	2.6.1.1	*Aat-3*	Eye	I
Creatine kinase	2.7.3.2	*Ck-1*	Muscle	I
		Ck-2	Muscle	I
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi-3*	Muscle	I
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3p-1*	Heart	III
		G3p-2	Heart	III
		G3p-3	Heart	III
Isocitrate dehydrogenase	1.1.1.42	*Idh-3,4*	Liver, eye	IV
Lactate dehydrogenase	1.1.1.27	*Ldh-1*	Muscle	I
		Ldh-3	Heart	I
		Ldh-4	Liver	I
		Ldh-5	Eye	I
Mannose-6-phosphate isomerase	5.3.1.8	*Mpi*	Heart, eye	II
Malate dehydrogenase	1.1.1.37	*Mdh-1,2*	Liver, muscle	III
		Mdh-3,4	Muscle	III
Peptidases	3.4.11/13			
Leucyl-glycyl-glycine activity		*Pep(Lgg)*	Muscle	I
Phenylalanyl-proline activity		*Pep(Pp-1)*	Heart, muscle	II
		Pep(Pp-2)	Heart, muscle	II
Glycyl-leucine activity		*Pep(Gl-1)*	Muscle	II
		Pep(Gl-2)	Eye	II
Phosphoglucomutase	5.4.2.2	*Pgm-2*	Muscle	I
Phosphogluconate dehydrogenase	1.1.1.44	*Pgd*	Liver	IV
Superoxide dismutase	1.15.1.1	*Sod-1*	Liver	II
		Sod-2	Liver	II

Hardy–Weinberg equilibrium expectations was examined using chi-square goodness-of-fit tests with a continuity correction of 0.25 (Emigh 1980). Pooling of genotypic frequencies eliminated classes with expected values less than four.

Variability observed at co-migrating duplicated loci (isoloci; Allendorf and Thorgaard 1984) was treated as if all variation appeared at one of the loci and the other locus was monomorphic. This treatment tends to give conservative estimates of genetic distances and heterozygosities.

Homogeneity of allelic frequencies among collections within a drainage, among drainages within a region, and among regions was examined using log-likelihood ratio (G-test) (Sokal and Rohlf 1981). When no heterogeneity was detected, the frequencies of the collections were pooled for subsequent analyses, since there was no reason to believe that the collections reflected genetically distinct populations. To examine the basis of the heterogeneity within a drainage, a stepwise test procedure (Sokal and Rohlf 1981) was used to partition the collections into groups within which no significant heterogeneity existed. Pooling of allelic frequencies eliminated classes with expected values less than two. Where multiple tests were performed, a correction for the level of significance was made (Cooper 1968). Levels of heterogeneity were compared using F-distributions: $F(\upsilon_1,\upsilon_2) = (G_1/\upsilon_1)/(G_2/\upsilon_2)$.

Gene diversity analysis (Nei 1973; Chakroborty 1980) was performed to decompose the genetic variability (heterozygosity) into hierarchical levels: $H_T = H_D + D_{DR} + D_{RT}$, where H_T is the total heterozygosity observed, H_D the average heterozygosity within drainages (or major subdivisions of drainages) within regions, $D_{DR} = H_R - H_D$ the variability attributable to diversity among drainages within regions, and $D_{RT} = H_T - H_R$ that among regions. Coefficients of gene differentiation (Nei 1973) were computed to estimate the proportion of total gene diversity due to each level of hierarchy.

Unbiased average heterozygosities and their sampling variances (Nei and Roychoudhury 1974; Nei 1978) were estimated using a FORTRAN program obtained from Nei and modified.

Relationships among the collections were examined from a maximum-likelihood evolutionary tree obtained using Felsenstein's (1973, 1984) CONTML program. This unrooted tree (network) is constructed from a chord measure of genetic distances (Cavalli-Sforza and Edwards 1967) by computing the topology with the maximum likelihood for the genetic distances between all pairs included in the data set. The program was run as recommended by the author.

Results

Of the 28 biochemical genetic loci examined (Table 2), 12 were monomorphic and identical in all collections; these were *Adh*, *Pgd*, *Pgm-2*, *Pep(Pp-1)*, *Ck-1* and *-2*, *Ldh-1*, *-3*, and *-5*, and *G3p-1*, *-2*, and *-3*. Six loci that had low levels of polymorphism (frequency of the common allele > 0.95 in all collections) were *Gpi-3*, *Ldh-4*, *Pep(G1-2)*, *Pep(Pp-2)*, *and isoloci Mdh-1,2*. Loci displaying more polymorphism (the frequency of the most common allele < 0.9 in at least one

TABLE 2. Biochemical genetic variation in collections of chinook salmon from drainages in Alas
are numbered as in Fig. 1 and grouped under designations for drainages and subdivisions of drainag

Group	Source of sample and year of collection	Loci												
		Mpi				*Sod-1*			*Mdh-3,4*				*Pep(G1-*	
		N	100	110	95	*N*	100	260	*N*	100	80	121	*N*	100
A	Copper River gillnet fishery													
	1. 1983	45	0.93	0.03	0.03	43	0.91	0.09	46	1.00	0.00	0.00	42	0.95
	2. 1984	102	0.93	0.07	0.00	102	0.89	0.11	102	1.00	0.00	0.00	99	0.94
B	Susitna River juveniles													
	3. Indian R. 1983	73	1.00	0.00	0.00	20	1.00	0.00	85	1.00	0.00	0.00	86	1.00
	4. Indian R. 1984	98	0.995	0.005	0.00	100	0.97	0.03	100	1.00	0.00	0.00	100	1.00
C	Bristol Bay gillnet fishery													
	5. Togiak 1983	106	0.90	0.10	0.00	84	0.93	0.07	109	0.99	0.00	0.01	109	0.995
	6. Dillingham 1983	46	0.91	0.04	0.04	—	—	—	95	0.99	0.00	0.01	96	1.00
D	Kuskokwim gillnet fishery													
	7. 1982	28	0.84	0.16	0.00	66	0.95	0.05	63	0.98	0.00	0.02	37	0.99
	8. 1983	110	0.91	0.09	0.00	114	0.97	0.03	105	0.99	0.00	0.01	108	0.995
	9. 1984	119	0.90	0.10	0.00	119	0.96	0.04	156	0.98	0.00	0.02	114	0.97
E	Unalakleet gillnet fishery													
	10. 1982	38	0.92	0.08	0.00	38	0.86	0.14	38	0.97	0.00	0.03	38	0.96
	11. 1983	25	0.94	0.06	0.00	27	0.91	0.10	27	0.96	0.04	0.00	27	0.98
F	Lower Yukon gillnet fishery													
	Subdivision I													
	12. Emmonak 1982	43	0.93	0.07	0.00	45	1.00	0.00	45	0.99	0.01	0.00	43	0.99
	13. Big Eddy 1982	122	0.92	0.08	0.00	116	0.97	0.03	132	0.98	0.02	0.004	107	0.97
	14. Big Eddy 1983	48	0.96	0.04	0.00	48	0.98	0.02	48	0.97	0.02	0.01	40	0.99
G	Subdivision II													
	15. Emmonak 1983	45	0.90	0.10	0.00	—	—	—	45	1.00	0.00	0.00	45	0.97
	16. St. Mary's 1983	96	0.94	0.06	0.005	80	0.99	0.01	85	0.97	0.01	0.02	98	1.00
H	Tanana River juveniles													
	17. L. Chena R. 1982	116	0.996	0.004	0.00	116	0.96	0.04	116	0.91	0.04	0.05	116	0.996
	18. U. Chena R. 1982	90	0.98	0.02	0.00	—	—	—	135	0.90	0.06	0.04	134	0.99
	19. L. Chena R. 1983	79	0.99	0.01	0.00	79	0.98	0.02	79	0.87	0.10	0.03	78	1.00
	20. Chatinika R. 1984	119	0.98	0.02	0.00	119	0.99	0.01	119	0.94	0.03	0.03	119	1.00
I	Chilkat River													
	21. Tahini R. 1983 egg take	34	0.62	0.38	0.00	34	0.84	0.16	34	1.00	0.00	0.00	34	1.00
	22. Tahini R. 1984 egg take	34	0.63	0.37	0.00	34	0.84	0.16	34	1.00	0.00	0.00	34	1.00
	23. Tahini R. 1984 juveniles	81	0.67	0.33	0.00	72	0.83	0.17	82	1.00	0.00	0.00	82	1.00
J	Taku River juveniles													
	24. Nahlin R. 1982	42	0.81	0.19	0.00	42	0.83	0.17	42	0.99	0.01	0.00	34	0.93
	25. L. Nakina 1982	85	0.76	0.24	0.00	73	0.80	0.20	90	0.94	0.00	0.06	84	0.91
	26. Mainstem 1981	100	0.82	0.18	0.00	98	0.92	0.08	100	0.98	0.02	0.00	100	0.88
	27. Mainstem 1982	92	0.78	0.19	0.03	95	0.90	0.10	95	0.97	0.03	0.00	85	0.95
	28. King Salmon Cr. 1982	47	0.77	0.23	0.00	47	0.92	0.08	47	1.00	0.00	0.00	47	0.89
K	Admiralty Island egg take													
	29. King Salmon R. 1983	64	0.94	0.06	0.00	21	0.95	0.05	64	1.00	0.00	0.00	64	0.78
	30. King Salmon R. 1984	64	0.95	0.05	0.00	64	0.96	0.04	64	1.00	0.00	0.00	64	0.88
	Stikine River													
L	Upper Stikine juveniles													
	31. Little Tahltan 1981	170	0.85	0.15	0.00	110	0.89	0.11	144	0.96	0.04	0.00	88	0.97
	32. Mainstem 1981	108	0.90	0.10	0.00	68	0.92	0.08	108	0.98	0.02	0.00	40	0.95
	33. Mainstem 1982	152	0.84	0.16	0.00	204	0.95	0.05	204	0.98	0.02	0.00	152	0.93
M	Lower Stikine egg take													
	34. Andrew Cr. 1982	125	0.73	0.27	0.00	116	0.94	0.06	126	0.98	0.02	0.00	119	0.86
N	Farragut River egg take													
	35. 1983	26	0.88	0.12	0.00	26	0.75	0.25	26	1.00	0.00	0.00	23	0.91
	36. 1984	24	0.92	0.08	0.00	24	0.81	0.19	24	1.00	0.00	0.00	23	1.00
O	Unuk River juveniles													
	37. 1982	82	0.82	0.18	0.00	82	0.87	0.13	82	0.98	0.02	0.00	82	0.98

s in Alaska. Allelic frequencies and collection sizes (N) for biochemical genetic loci. Collections
f drainages. Alleles are designated by their mobility relative to the most common allele (100).

	Pep(G1-1)			*Pep(Lgg)*				*Gpi-3*				*Mdh-1,2*				*Ldh-4*		
N	100	90	*N*	100	130	95	*N*	100	105	93	*N*	100	120	82	*N*	100	117
12	0.95	0.05	45	0.97	0.03	0.00	46	1.00	0.00	0.00	46	1.00	0.00	0.00	45	1.00	0.00
99	0.94	0.06	102	0.95	0.05	0.00	102	0.995	0.005	0.00	102	1.00	0.00	0.00	80	1.00	0.00
86	1.00	0.00	79	0.97	0.03	0.00	86	1.00	0.00	0.00	86	1.00	0.00	0.00	86	1.00	0.00
00	1.00	0.00	100	0.91	0.09	0.00	100	1.00	0.00	0.00	100	1.00	0.00	0.00	100	1.00	0.00
09	0.995	0.005	106	0.91	0.06	0.03	109	1.00	0.00	0.00	193	1.00	0.00	0.00	109	1.00	0.00
96	1.00	0.00	91	0.94	0.05	0.01	55	1.00	0.00	0.00	95	1.00	0.00	0.00	40	1.00	0.00
37	0.99	0.01	12	0.88	0.12	0.00	50	1.00	0.00	0.00	63	1.00	0.00	0.00	68	1.00	0.00
08	0.995	0.005	84	0.90	0.09	0.01	104	1.00	0.00	0.00	105	1.00	0.00	0.00	105	0.995	0.005
14	0.97	0.03	112	0.87	0.10	0.03	119	0.996	0.004	0.00	119	1.00	0.00	0.00	119	0.996	0.004
38	0.96	0.04	36	0.96	0.04	0.00	38	1.00	0.00	0.00	38	1.00	0.00	0.00	38	1.00	0.00
27	0.98	0.02	27	0.93	0.04	0.04	27	1.00	0.00	0.00	27	1.00	0.00	0.00	27	1.00	0.00
43	0.99	0.01	29	0.76	0.24	0.00	—	—	—	—	45	1.00	0.00	0.00	45	0.98	0.02
07	0.97	0.03	95	0.79	0.20	0.01	81	1.00	0.00	0.00	133	1.00	0.00	0.00	131	0.99	0.01
40	0.99	0.01	44	0.91	0.07	0.02	46	1.00	0.00	0.00	48	1.00	0.00	0.00	41	1.00	0.00
45	0.97	0.03	44	0.92	0.07	0.01	45	1.00	0.00	0.00	45	1.00	0.00	0.00	45	1.00	0.00
98	1.00	0.00	76	0.91	0.05	0.05	97	1.00	0.00	0.00	85	1.00	0.00	0.00	98	1.00	0.00
16	0.996	0.004	116	0.80	0.20	0.00	34	1.00	0.00	0.00	116	1.00	0.00	0.00	116	1.00	0.00
34	0.99	0.01	—	—	—	—	45	1.00	0.00	0.00	135	1.00	0.00	0.00	135	1.00	0.00
78	1.00	0.00	—	—	—	—	79	1.00	0.00	0.00	79	0.98	0.00	0.02	79	1.00	0.00
19	1.00	0.00	119	0.74	0.26	0.00	119	1.00	0.00	0.00	119	1.00	0.00	0.00	119	1.00	0.00
34	1.00	0.00	34	0.96	0.04	0.00	34	0.97	0.03	0.00	34	1.00	0.00	0.00	34	1.00	0.00
34	1.00	0.00	34	0.88	0.12	0.00	34	0.98	0.02	0.00	34	1.00	0.00	0.00	34	1.00	0.00
82	1.00	0.00	82	0.92	0.08	0.00	82	0.96	0.04	0.00	82	1.00	0.00	0.00	82	1.00	0.00
34	0.93	0.07	42	0.96	0.04	0.00	42	0.96	0.04	0.00	42	1.00	0.00	0.00	42	1.00	0.00
84	0.91	0.09	90	1.00	0.00	0.00	50	0.99	0.01	0.00	90	1.00	0.00	0.00	90	1.00	0.00
00	0.88	0.12	100	0.98	0.02	0.01	100	0.96	0.04	0.00		—	—	—	—	—	—
85	0.95	0.05	59	1.00	0.00	0.00	95	0.99	0.01	0.00	95	1.00	0.00	0.00	95	1.00	0.00
47	0.89	0.11	44	0.98	0.02	0.00	47	1.00	0.00	0.00	47	1.00	0.00	0.00	47	1.00	0.00
64	0.78	0.22	60	1.00	0.00	0.00	64	1.00	0.00	0.00	64	1.00	0.00	0.00	64	1.00	0.00
64	0.88	0.12	64	0.98	0.02	0.00	64	1.00	0.00	0.00	64	1.00	0.00	0.00	64	1.00	0.00
88	0.97	0.03	—	—	—	—	140	1.00	0.00	0.00	—	—	—	—	—	—	—
40	0.95	0.05	68	0.98	0.02	0.00	108	0.99	0.01	0.00	—	—	—	—	—	—	—
52	0.93	0.07	102	0.995	0.005	0.00	152	0.97	0.03	0.00	204	1.00	0.00	0.00	152	1.00	0.00
19	0.86	0.14	116	0.97	0.03	0.00	93	0.97	0.03	0.00	126	1.00	0.00	0.00	126	0.996	0.004
23	0.91	0.09	26	1.00	0.00	0.00	26	1.00	0.00	0.00	26	1.00	0.00	0.00	26	1.00	0.00
23	1.00	0.00	23	0.98	0.02	0.00	24	0.98	0.02	0.00	24	1.00	0.00	0.00	24	1.00	0.00
82	0.98	0.02	54	1.00	0.00	0.00	40	0.99	0.01	0.00	82	1.00	0.00	0.00	82	1.00	0.00

TABLE 2. (*Concluded*)

Group	Source of sample and year of collection	*Pep(G1-2)*				*Sod-2*			*Aat-3*				
		N	100	70	105	*N*	100	142	*N*	100	85	*N*	10
A	Copper River gillnet fishery												
	1. 1983	42	1.00	0.00	0.00	43	1.00	0.00	45	0.68	0.32	—	—
	2. 1984	99	1.00	0.00	0.00	79	0.96	0.04	100	0.77	0.23	84	0.9
B	Susitna River juveniles												
	3. Indian R. 1983	86	1.00	0.00	0.00	—	—	—	75	0.86	0.14	81	1.0
	4. Indian R. 1984	100	1.00	0.00	0.00	92	0.92	0.08	—	—	—	82	1.0
C	Bristol Bay gillnet fishery												
	5. Togiak 1983	109	1.00	0.00	0.00	83	0.92	0.08	109	0.88	0.12	106	0.9
	6. Dillingham 1983	96	1.00	0.00	0.00	73	0.95	0.05	93	0.92	0.08	95	0.9
D	Kuskokwim gillnet fishery												
	7. 1982	37	1.00	0.00	0.00	16	0.91	0.09	—	—	—	—	—
	8. 1983	103	1.00	0.00	0.00	112	0.95	0.05	105	0.86	0.14	65	1.0
	9. 1984	119	1.00	0.00	0.00	156	0.93	0.07	105	0.83	0.17	100	0.9
E	Unalakleet gillnet fishery												
	10. 1982	38	1.00	0.00	0.00	37	0.96	0.04	—	—	—	—	—
	11. 1983	27	1.00	0.00	0.00	26	0.94	0.068	25	0.86	0.14	27	0.9
	Lower Yukon gillnet fishery												
F	Subdivision I												
	12. Emmonak 1982	45	1.00	0.00	0.00	35	0.93	0.07	—	—	—	—	—
	13. BigEddy 1982	133	1.00	0.00	0.00	109	0.95	0.05	—	—	—	—	—
	14. Big Eddy 1983	41	1.00	0.00	0.00	41	0.90	0.10	48	0.92	0.08	38	0.9
G	Subdivision II												
	15. Emmonak 1983	45	1.00	0.00	0.00	45	0.96	0.04	45	0.81	0.19	45	1.0
	16. St. Mary's 1983	98	0.995	0.005	0.00	89	0.90	0.10	95	0.76	0.24	86	0.9
H	Tanana River juveniles												
	17. L. Chena R. 1982	116	1.00	0.00	0.00	36	0.92	0.08	—	—	—	—	—
	18. U. Chena R. 1982	134	1.00	0.00	0.00	—	—	—	—	—	—	—	—
	19. L. Chena R. 1983	78	1.00	0.00	0.00	11	1.00	0.00	—	—	—	41	1.0
	20. Chatinika R. 1984	119	1.00	0.00	0.00	—	—	—	117	0.70	0.30	97	1.0
I	Chilkat River												
	21. Tahini R. 1983 egg take	18	1.00	0.00	0.00	34	1.00	0.00	32	0.93	0.07	34	1.0
	22. Tahini R. 1984 egg take	34	1.00	0.00	0.00	34	1.00	0.00	34	0.94	0.06	34	0.9
	23. Tahini R. 1984 juveniles	82	1.00	0.00	0.00	—	—	—	81	0.96	0.04	65	0.9
J	Taku River juveniles												
	24. Nahlin R. 1982	39	0.97	0.00	0.03	42	1.00	0.00	—	—	—	—	—
	25. L. Nakina 1982	89	1.00	0.00	0.00	—	—	—	—	—	—	90	1.0
	26. Mainstem 1981											100	0.8
	27. Mainstem 1982	85	1.00	0.00	0.00	95	1.00	0.00	46	0.95	0.05	14	1.0
	28. King Salmon Cr. 1982	47	1.00	0.00	0.00	—	—	—	—	—	—	47	1.0
K	Admiralty Island egg take												
	29. King Salmon R. 1983	63	1.00	0.00	0.00	21	1.00	0.00	51	1.00	0.00	40	1.0
	30. King Salmon R. 1984	64	1.00	0.00	0.00	64	1.00	0.00	44	0.98	0.02	53	1.0
	Stikine River												
L	Upper Stikine juveniles												
	31. Little Tahltan 1981	—	—	—	—	—	—	—	—	—	—	—	—
	32. Mainstem 1981	—	—	—	—	—	—	—	—	—	—	108	0.9
	33. Mainstem 1982	50	1.00	0.00	0.00	204	1.00	0.00	147	0.90	0.10	140	1.0
M	Lower Stikine egg take												
	34. Andrew Cr. 1982	126	1.00	0.00	0.00	69	1.00	0.00	33	0.83	0.17	68	0.8
N	Farragut River egg take												
	35. 1983	23	1.00	0.00	0.00	26	1.00	0.00	—	—	—	26	1.0
	36. 1984	24	1.00	0.00	0.00	24	1.00	0.00	23	1.00	0.00	22	1.0
O	Unuk River juveniles												
	37. 1982	82	1.00	0.00	0.00	—	—	—	40	0.95	0.05	82	1.0

	Idh-3,4						*Pep(Pp-2)*				Sample sizes of monomorphic loci											
	N	100	136	74	127	50	*N*	100	110	105	*Ldh-3*	*Ck-1*	*Ck-2*	*Ldh-5*	*G3p-1*	*G3p-2*	*Pgm-2*	*Pgd*	*G3p-3*	*Pep (Pp-1)*	*Ldh-1*	*Adh*
	—	—	—	—	—	—	46	1.00	0.00	0.00	45	34	34	45	43	43	43	41	43	46	46	35
	84	0.994	0.006	0.00	0.00	0.00	80	1.00	0.00	0.00	81	102	102	102	80	80	97	101	80	80	102	74
	81	1.00	0.00	0.00	0.00	0.00	86	0.994	0.006	0.00	86	77	77	86	86	86	86	86	86	86	86	76
	82	1.00	0.00	0.00	0.00	0.00	100	1.00	0.00	0.00	100	100	100	100	100	100	100	97	100	100	100	60
	106	0.995	0.005	0.00	0.00	0.00	109	1.00	0.00	0.00	193	25	25	109	84	84	109	72	84	109	109	108
	95	0.995	0.005	0.00	0.00	0.00	46	0.99	0.01	0.00	40	87	87	102	72	72	96	97	72	46	95	105
	—	—	—	—	—	—	58	0.99	0.00	0.01	65	57	57	68	63	63	65	—	63	58	65	43
	65	1.00	0.00	0.00	0.00	0.00	101	1.00	0.00	0.00	105	98	98	105	112	112	104	87	112	101	104	102
	100	0.98	0.02	0.00	0.00	0.00	115	1.00	0.00	0.00	119	119	119	119	119	119	119	87	119	115	119	76
	—	—	—	—	—	—	38	0.97	0.03	0.00	38	37	37	38	38	38	38	38	38	38	38	38
	27	0.98	0.02	0.00	0.00	0.00	27	1.00	0.00	0.00	27	—	—	27	25	25	27	25	25	27	27	27
	—	—	—	—	—	—	45	1.00	0.00	0.00	45	—	—	45	45	45	45	45	45	45	45	45
	—	—	—	—	—	—	112	0.996	0.00	0.004	131	102	102	127	80	80	107	35	80	112	133	81
	38	0.961	0.039	0.00	0.00	0.00	41	1.00	0.00	0.00	45	27	27	48	41	41	41	42	41	41	48	41
	45	1.00	0.00	0.00	0.00	0.00	45	1.00	0.00	0.00	45	27	27	45	—	—	45	23	—	45	45	38
	86	0.994	0.006	0.00	0.00	0.00	83	1.00	0.00	0.00	98	45	45	98	75	75	86	87	75	94	85	101
	—	—	—	—	—	—	78	1.00	0.00	0.00	116	76	76	40	116	116	76	—	116	78	116	—
	—	—	—	—	—	—	135	1.00	0.00	0.00	135	135	135	135	45	45	135	—	45	135	135	—
	41	1.00	0.00	0.00	0.00	0.00	79	1.00	0.00	0.00	79	79	79	79	77	77	28	41	77	79	79	73
	97	1.00	0.00	0.00	0.00	0.00	119	1.00	0.00	0.00	119	118	118	119	119	119	119	119	119	119	119	119
	34	1.00	0.00	0.00	0.00	0.00	34	1.00	0.00	0.00	34	16	16	34	34	34	16	34	34	34	34	26
	34	0.93	0.00	0.07	0.00	0.00	34	1.00	0.00	0.00	34	31	31	34	34	34	34	34	34	34	34	34
	65	0.92	0.00	0.08	0.00	0.00	81	1.00	0.00	0.00	82	82	82	82	82	82	82	81	82	82	62	82
	—	—	—	—	—	—	39	1.00	0.00	0.00	42	42	42	42	42	42	—	42	42	42	42	42
	90	1.00	0.00	0.00	0.00	0.00	50	0.99	0.01	0.00	90	90	90	—	90	90	—	90	90	45	90	90
	100	0.89	0.01	0.04	0.06	0.00	—	—	—	—	—	—	—	—	—	—	100	—	—	—	—	—
	14	1.00	0.00	0.00	0.00	0.00	91	1.00	0.00	0.00	95	85	85	95	95	95	45	94	95	93	95	90
	47	1.00	0.00	0.00	0.00	0.00	47	0.96	0.04	0.00	47	47	47	42	47	47	47	45	47	47	47	47
	40	1.00	0.00	0.00	0.00	0.00	64	1.00	0.00	0.00	—	—	64	64	64	64	64	64	64	64	64	64
	53	1.00	0.00	0.00	0.00	0.00	64	1.00	0.00	0.00	64	59	59	64	64	64	64	59	64	64	64	64
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	170	—	—	—	—	—
	108	0.96	0.00	0.04	0.00	0.00	—	—	—	—	—	—	—	—	—	108	—	—	—	—	—	—
	140	1.00	0.00	0.00	0.00	0.00	151	1.00	0.00	0.00	102	130	130	152	142	142	152	90	142	152	102	142
	68	0.89	0.11	0.00	0.00	0.00	67	1.00	0.00	0.00	126	126	126	126	126	126	109	81	126	125	126	92
	26	1.00	0.00	0.00	0.00	0.00	26	1.00	0.00	0.00	26	24	24	26	25	25	25	26	25	26	26	22
	22	1.00	0.00	0.00	0.00	0.00	24	1.00	0.00	0.00	24	24	24	20	24	24	—	24	24	24	24	24
	82	1.00	0.00	0.00	0.00	0.00	78	1.00	0.00	0.00	82	56	56	82	82	82	—	42	82	82	82	82

TABLE 3. Heterogeneity of Alaskan chinook salmon. Log-likelihood ratio analysis (Sokal and Rohlf 1981) of allelic frequencies. Group designations refers to drainages or subdivisions within drainages (see Table 2). *No test* indicates presence of a single collection. *$P<0.001$; $P<0.05$ for all others.

Group designation	Region/drainage	Heterogeneity within drainages		Heterogeneity in drainage subdivisions		Heterogeneity within regions	
		G	df	*G*	df	*G*	df
	South-central Alaska					85.94	6*
A	Copper River	3.17	4				
B	Susitna River	5.37	1				
	Western Alaska					305.14	30*
C	Bristol Bay	3.87	4				
D	Kuskokwim River	3.68	5				
E	Unalakleet River	0.82	1				
	Yukon River	106.14	17*				
F	Lower river I			12.27	6		
G	Lower river II			5.13	4		
H	Tanana River			18.90	9		
	Southeastern Alaska					375.21	36*
I	Chilkat River	13.28	8				
J	Taku River	25.56	12				
K	Admiralty Island	5.09	2				
	Stikine River	93.21	15*				
L	Upper river			18.42	8		
M	Lower river			*No test*			
N	Farragut River	0.86	2				
O	Unuk River	*No test*					
	Heterogeneity among regions					956.66	14*

collection) were *Mpi*, *Sod-1* and *-2*, *Pep(G1-1)*, *Pep(Lgg)*, *Aat-3*, and isoloci *Mdh-3,4* and *Idh-3,4*. No deviations from Hardy–Weinberg equilibrium expectations were observed ($P > 0.05$); however, only large deviations would be detectable given the collection sizes.

For most drainages, collections were made at more than one site and/or in more than one year to examine spatial and temporal heterogeneity existing within each drainage. After correction for multiple testing (Cooper 1968), heterogeneity was observed only in the Stikine and Yukon ($P < 0.001$) (Table 3). No spatial or temporal variability was observed in the other systems.

To examine the nature of the heterogeneity within the Yukon and Stikine drainages, a stepwise test procedure (Sokal and Rohlf 1981) was used to partition the collections within each drainage into groups within which no significant heterogeneity existed. The Yukon collections produced three groups, two groups of lower Yukon gillnet and test fishery collections (Subdivisions I and II) and the collections from the Tanana River tributaries (Table 2). The Tanana collections were juveniles taken near their natal grounds and probably reflect one or a very few populations. In contrast, the lower Yukon collections were taken from the particular mixture of populations passing through the fishery at the time of capture.

Heterogeneity within the Stikine River was attributable to the collection taken from Andrew Creek, near the mouth of the Stikine River, suggesting that divergence has occurred between upstream and downstream chinook populations. All subsequent analyses were performed using data pooled from a drainage or from groups of collections minimizing heterogeneity within a drainage (Table 3).

The drainages or significant subdivisions of the three major geographic regions, western, south-central, and southeastern Alaska, were further examined using log-likelihood ratio analysis to determine the extent of divergence within and among regions (Table 3). Significant heterogeneity ($P < 0.001$) was observed at both levels of hierarchy; however, relatively more ($P < 0.01$) was observed among regions than within regions.

Gene diversity analysis was used to partition the variation observed at 28 genetic loci into components attributable to regional differences, to differences among significant subdivisions within regions, and to the average amount observed within drainages (or their subdivisions). The Unuk River collection was not included in this analysis because data were not available for two of the loci. Most of the variability observed was expressed within drainages (94.09%). Divergence within regions accounted for 3.32% of the variability and differences among regions for 2.59% (Table 4).

To examine the genetic relationships of Alaskan chinook and chinook from other more southerly drainages, an unrooted maximum-likelihood tree (Felsenstein 1973, 1984) was constructed from allelic frequencies of 23 loci common to our data and to four groups of southern populations previously reported by Milner et al. (1983). The loci not used had little or no polymorphism; they were *Pep(Pp-1)*, *Pep(Pp-2)*, *Ldh-1*, *Adh*, and *G3p-3*. Southern collections used included spring-run and fall-run wild populations chosen as representative of two life history types. Unweighted means of the allelic frequencies of chinook from Nooksack and Soleduc rivers in Washington are referred to as Washington (spring). Similarly, allelic frequencies of chinook from Hoh and Queets rivers in Washington were combined as Washington (fall). Data from three Fraser

TABLE 4. Gene diversity analysis of Alaskan chinook salmon using 28 loci.

Source	Gene diversity	Coefficient of gene differentiation
Average within drainages and subdivisions	H_D = 0.03267	H_D/H_T = 0.9409
Average among drainages within regions	D_{DR} = 0.00115	G_{DR} = 0.0332
Among regions	D_{RT} = 0.00090	G_{RT} = 0.0259
Total gene diversity	H_T = 0.03472	

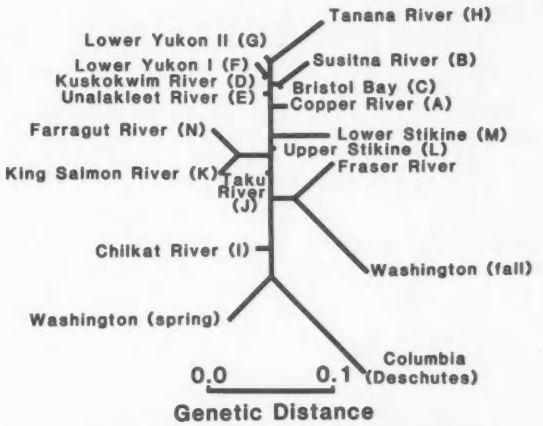

FIG. 2. Phenetic relationships among Alaskan chinook and other more southerly groups of chinook. The maximum-likelihood method of Felsenstein (1973, 1984) was employed to estimate this unrooted tree using data from 23 loci. The tree is oriented to approximate the geographical relationships among the collections. The distances between nodes and collections are chord measure genetic distances (Cavalli-Sforza and Edwards 1967). Angles are arbitrary. Data for non-Alaskan collections are from Milner et al. (1983).

River collections were pooled in the same way and included because of their proximity to southeastern Alaska. Spring-run chinook from Warmsprings on the Deschutes River, a Columbia River tributary, were chosen because of their geographical distance from all other collections.

Western Alaskan and southeastern Alaskan chinook are clearly separated from each other (Fig. 2). Chinook from one south-central drainage, Susitna River (B), were more closely related to chinook from western Alaska, while the Copper River (A) fish were intermediate between western and southeastern collections but more similar to western Alaskan chinook. Other trees generated from the CONTML program were similar to the maximum-likelihood tree presented. Only details of the relationships within the southeastern Alaskan region or within the western Alaskan region varied among the less likely trees produced.

Western Alaskan Chinook

Two observations may be made from the genetic relationships among the chinook salmon from western Alaskan drainages. Although heterogeneity exists among most of the drainages, the overall genetic differentiation among the different populations was small for the 28 loci considered in this study. Secondly, the chinook in the Tanana drainage, tributaries of the upper Yukon (H, Fig. 1), are unique. While no fixed differences were observed in the Tanana collections, *Mpi* variability was lower and *Mdh-3,4* higher than observed among any of the other drainages. Similar results were obtained using other measures of genetic distances (Rogers 1972; Nei 1978) and other clustering algorithms (results not shown). Western Alaskan collections were generally more variable at *Pep(Lgg)*, *Sod-2*, and *Aat-3*. The average heterozygosity per locus of these collections is low, 0.032 ± 0.006 (mean ± SE).

Southeastern Alaskan Chinook

Analysis of southeastern Alaskan collections (Fig. 2) indicated more differentiation among drainages than was observed in western Alaska. The greater extent of differentiation is paralleled by a small increase ($P < 0.05$) in variation of the average heterozygosity per locus (0.033 ± 0.012) among southeastern Alaskan collections. Southeastern Alaskan collections generally showed more polymorphism at *Mpi*, *Sod-1*, and *Gpi-3*. By excluding data from *Sod-2* and *Pgm-1*, the Unuk River collection was included in the analysis (not shown). Unuk River chinook were most similar to the Sikine and Taku collections. Similar results were obtained using other genetic distance measures (Rogers 1972; Nei 1978) and other clustering algorithms (results not shown).

The genetic compositions of most of the southeastern Alaskan chinook populations were intermediate between those of western Alaskan chinook and of Washington fall-run (Queets and Hoh) and Fraser River chinook (Fig. 2). An exception is Chilkat River chinook, which differ from other southeastern Alaskan collections and which branch between the branch for the Fraser River and Washington fall-run (Queets and Hoh) and the branch for Washington spring-run (Soleduc and Nooksack) chinook. Some of the uniqueness of the Chilkat fish was due to more variability at the *Mpi* locus.

Discussion

Fisheries Management Implications

With the exception of the Yukon and Stikine rivers, no significant heterogeneity was observed within Alaskan drainages; however, small but statistically significant differences did exist among most of the drainages, indicating that no substantial straying among drainages takes place; otherwise, the regions would be homogeneous. The absence of heterogeneity among collections made within a drainage in different years suggests that the genetic compositions of chinook are temporally stable. Similarly, no spatial heterogeneity was

observed within the Tanana, Bristol Bay, Chilkat, or Taku drainages or in the upper Stikine River tributaries. Of course, the degree of differentiation among collections taken to examine spatial and temporal variation may have been too small to measure.

Several collections were taken from gillnet and test fisheries which often exploit mixtures of populations. The conformity with Hardy–Weinberg expectations and the absence of heterogeneity within the Unalakleet, Kuskokwim, and Copper River drainages and within Bristol Bay suggest that little difference exists in the genetic compositions of the populations comprising these fisheries. Such small divergence among stocks would preclude the use of biochemical genetics as a management tool for these fisheries. Tests for Hardy–Weinberg frequencies are weak, however, and additional loci which reflect divergence may exist.

One of the exceptions to the overall genetic similarity of collections within a drainage was the Yukon system. This heterogeneity is not surprising. The Yukon River is comparable in size to the Columbia River in which considerable differentiation of chinook populations has taken place (Milner et al. 1981). The collections taken from gillnet and test fisheries at the Yukon River mouth probably reflect mixtures of a number of such populations. The heterogeneity observed among these collections (Table 3) shows that the composition of stocks passing through the fishery varies during the spawning run.

Collections from the Tanana system were composed of juveniles; and while some mixing among local populations may have occurred, our results indicate that the contributing populations are closely related. The Tanana River enters the Yukon in central Alaska near Fairbanks; the Yukon River, however, extends much further into Canada. Because of the size of the Yukon and remoteness of many tributaries, we were unable to obtain samples either from adults or from emerging juveniles near their natal grounds. The heterogeneity observed within the Yukon suggests that biochemical genetic methods may be applicable to management of Yukon fisheries if adequate baseline information can be obtained. The existence of one genetically distinct group of chinook salmon in the Yukon suggests that others may exist. The heterogeneity among lower Yukon gillnet and test fishery collections also indicates that mixtures of distinct populations had been sampled.

Among southeastern Alaskan chinook, more genetic divergence was observed than among those from western Alaskan drainages. The differences in degree of differentiation were not reflected by average heterozygosities of the two regions. Differences also exist between southeastern Alaskan and more southerly populations. Biochemical genetic methods can be useful to fisheries management as a stock separation tool. Whether the variability we have observed is sufficient to resolve components of mixed Alaskan stocks or to separate Alaskan and non-Alaskan stocks will require simulation studies (e.g. Beacham et al. 1985).

Genetic variability is a valuable resource for brood stock development. In southeastern Alaska, many of the drainages are genetically distinct and may have unique characteristics advantageous to culturists. For example, the difference between the upstream and downstream populations of chinook in the Stikine and possibly Taku may be pertinent to selection of brood stock. Some of the genetic differences may have resulted from natural selection, and could be important for brood stock development. The current practice of using only coastal populations of chinook salmon to develop brood stock for coastal hatcheries appears genetically justified. The existence of genetic variability among southeastern Alaskan chinook provides an opportunity for future brood stock development and culture of chinook salmon. The few wild stocks of chinook that remain are relatively small in size and fragile. These valuable genetic resources must be responsibly managed and conserved.

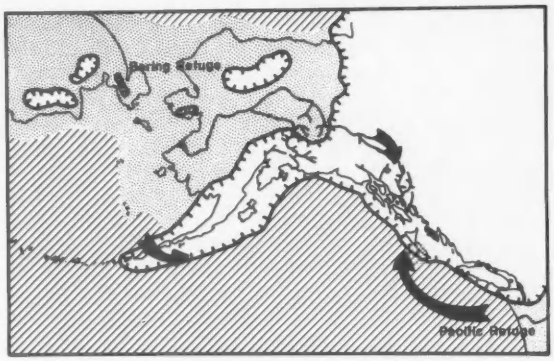

FIG. 3. Proposed movements of chinook salmon colonizing southeastern Alaska from the Bering and Pacific refuges following Wisconsin glaciation. Stipled areas represent land exposed at the peak of Wisconsin glaciation; diagonally striped areas represent oceans. The present coastline and locations of portions of drainages possibly involved are presented. (Figure after Karlstrom 1961; McPhail and Lindsey 1970; Hamilton and Thorson 1983.)

Origin of Southeastern Alaskan Chinook

Chinook salmon emerged as a species and dispersed prior to the last major glaciation, the Wisconsin (McPhail and Lindsey 1970). At the peak of the Wisconsin, the north Pacific coast from southern British Columbia to the Alaskan Peninsula was ice covered (Fig. 3). Following the recession of the glaciers, river systems adequate to support chinook salmon were probably colonized by fish surviving in either the Bering or Pacific refuges (McPhail and Lindsey 1970).

The unrooted tree derived from biochemical genetic characters (Fig. 2) suggests that the Chilkat River populations are most closely related to the southern populations. The genetic compositions of the other southeastern Alaskan populations, however, appear to have been influenced by chinook from western Alaska as well as by southern chinook populations. It is interesting that the southern stocks which appear most closely related to the spring-run, stream-type southeastern Alaskan populations are Fraser River (summer-run and both stream- and ocean-type) and Washington fall-run chinook (ocean-type). These observations suggest that life history patterns may be selected for after colonization; but, because of the limited comparisons made with southern populations, the paucity of data available from wild British Columbia chinook populations, and limitations in choice of loci, further conjecture on this relationship would not be productive.

The spread of chinook from river to river along the coast northward from the Pacific Refuge is plausible (Fig.3). A similar mode of dispersal from the Bering Refuge, along a much longer coastline which has fewer suitable rivers, seems less likely. A simpler means by which western Alaskan (specifically Yukon River) chinook could have colonized south-

eastern Alaskan streams is headwater capture. The headwaters of the Taku and Stikine rivers are in close geographical proximity to each other and to the headwaters of the Yukon. Geological evidence indicates that glaciers from the persistent ice cap periodically advance and block the Taku causing it to back up into the Yukon drainage (Kerr 1948) in a manner similar to the recent advance of Hubbard Glacier across Russell Fjord. That such events have affected distribution of fishes is evidenced by the presence in the Taku headwaters of primary freshwater fish including northern pike (*Esox lucius*), lake trout (*Salvelinus namaycush*), and Arctic grayling (*Thymallus arcticus*) as well as other freshwater species that most likely originated from the Yukon (Lindsey 1975). Introductions of Yukon River chinook into the Taku drainage may have followed episodes during which the Taku drainage was blocked (Fig. 3). These populations could have subsequently interacted with populations derived from the Pacific Refuge. Other southeastern Alaskan transmountain rivers, Alsek and Chilkat, also possess primary freshwater fish and may have been involved in colonization of salmonid populations via the Yukon River (Lindsey 1975).

Our proposal for post-Wisconsin colonization of southeastern Alaskan rivers by chinook salmon is based upon their genetic relationships but also requires the assumption that chinook salmon survived the Wisconsin glaciation in both Bering and Pacific refuges (McPhail and Lindsey 1970). If chinook survived in only one refuge, the larger variability in both biochemical genetic compositions and life histories found among southern populations suggests that the Pacific Refuge is the more likely of the two because more recently founded populations usually have less genetic variability (e.g. Gharrett and Thomason 1987). If chinook did not survive in the Bering Refuge, all Alaskan rivers may have been colonized from the Pacific Refuge and/or possibly from refuges in Asia. Unfortunately, little is known about the genetic composition of Asian chinook populations. The similarities shared by southeastern and western Alaskan chinook and the logic presented above, however, make it seem likely that the transmountain rivers in southeastern Alaska with headwaters near those of the Yukon River would have been important in colonizing the Yukon River and, subsequently, the rest of south-central and western Alaska.

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Chlorophyll–Phosphorus Relationships for Subarctic Lakes in Western Canada

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Ostrofsky, M. L., and F. H. Rigler. 1987. Chlorophyll–phosphorus relationships for subarctic lakes in western Canada. Can. J. Fish. Aquat. Sci. 44: 775–781.

Concentrations of total phosphorus and chlorophyll *a* were measured weekly in 49 lakes in the vicinity of Yellowknife, N.W.T., Canada, from May to September 1977. The relationship between [TP_{spr}] and [Chl a_{sm}] was significantly different from the Dillon–Rigler model, but similar to relationships developed for lakes in Alberta, British Columbia, and the Yukon Territory. Empirical relationships were developed between [TP_{spr}] and maximum chlorophyll *a* concentrations and the probability of exceeding critical concentrations of chlorophyll *a*. These models may be potentially more useful than models which predict only a mean summer chlorophyll concentration.

De mai à septembre 1977, on a quantifié hebdomadairement les concentrations de phosphore total et de chlorophylle *a* dans 49 lacs près de Yellowknife (T.N.O.), Canada. La relation entre [PT] au printemps et [Chl *a*] en été était nettement différente du modèle de Dillon–Rigler mais semblable aux relations déterminées pour des lacs de l'Alberta, de la Colombie-Britannique et du Yukon. On a élaboré des relations empiriques entre, d'une part, la [PT] au printemps et les concentrations maximums de chlorophylle *a* et, d'autre part, la probabilité d'excéder les concentrations critiques de chlorophylle *a*. Ces modèles peuvent être plus utiles que les modèles qui ne prédisent que la concentration estivale moyenne de chlorophylle.

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Models that predict the summer concentration of chlorophyll *a* from spring total phosphorus have been widely used to predict the impact of nutrient increases or decreases on a wide variety of lakes, generally from temperate North America and Europe. The utility of these models outside this restricted geographic region is unknown. With the increasing use of subarctic regions for energy and resource extraction, and recreation, the problem of lake eutrophication has become increasingly widespread. Management of lakes in subarctic regions requires a collection of tools or models which will enable resource managers to predict the effect of increasing nutrient supply to these lakes. Such models would allow a rational approach to the exploitation of subarctic regions, since some of the consequences of development could be predicted.

In 1976, we began a study to determine the applicability of a sequence of models, developed in more temperate regions, to assess the impact of changes in phosphorus loading to lakes in two regions of the Canadian subarctic. The sequence of models examined was essentially that of Dillon and Rigler (1975), which predicts the phosphorus loading to a lake from a knowledge of its drainage basin characteristics. Phosphorus retention is predicted from the hydrologic characteristics of the lake; retention and phosphorus loading are then used to predict the spring total phosphorus concentration. From total phosphorus concentration, summer average chlorophyll concentration and ultimately Secchi transparency in the lake may be predicted.

Parts of this project have already been reported. Ostrofsky (1978a) has examined the retention of phosphorus in 53 lakes near Yellowknife, N.W.T., Canada, and Smith et al. (1984) have summarized the results of phosphorus enrichment of lakes near Schefferville, Que. This contribution presents the results of our examination of chlorophyll *a* concentrations in 49 lakes near Yellowknife.

Methods

We selected for study a collection of lakes with a broad range of phosphorus concentrations. Previous studies in the subarctic have often been hampered by a very narrow range in lake characteristics, making empirical modelling difficult. Since existing empirical models often show large residual variation, possibly due in part to methodological differences, we also used consistent procedures for all analyses from all lakes.

Three criteria were used in the selection of lakes for study: easy accessibility to facilitate a regular sampling schedule, wide variation in trophic status, preferably spanning an order of magnitude in phosphorus concentrations, and spring total nitrogen to total phosphorus ratios > 12 to satisfy the assumption of phosphorus limitation (Dillon and Rigler 1974). Based on these criteria, we selected 49 lakes in the vicinity of Yellowknife. These lakes ranged in size from 4 to 885 ha in

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TABLE 1. Physical and chemical characteristics of the 49 study lakes.

Lake	Area (ha)	Alkalinity ($mg \cdot L^{-1}$)	Conductivity ($\mu mho \cdot cm^{-1}$)	pH
Lake 3	13	45	113	7.5
Lake 6	15	48	115	7.3
Lake 5	9	87	176	8.3
Fiddlers L. S.	46	54	115	8.3
Fiddlers L. N.	33	39	80	7.7
Fox	58	76	172	8.1
Long	115	44	125	7.6
Stock	51	62	146	7.8
Frame	93	108	260	8.1
Grace	63	41	116	7.7
Lake 1	22	69	163	8.4
Daigle	13	126	295	8.1
Finger	4	84	243	8.3
Shadow	9	93	224	8.3
X	7	105	299	7.9
Rater	23	107	370	7.9
Walsh	885	104	218	8.3
Vee	71	113	225	8.0
David	14	82	315	8.0
Gold	4	104	285	7.9
Gar	34	49	170	7.6
Trapper	28	45	225	7.9
Tibbit L. N.	149	20	48	7.4
Tibbit L. S.	202	22	48	7.5
Lake 50	10	135	230	8.8
Peninsula	86	73	125	8.2
Lake 49	24	47	95	7.9
Lake 48	9	50	104	7.7
Reid	563	24	53	7.5
Pickerel	161	46	95	7.7
Lake 47	14	43	98	7.2
Lake 46	16	109	200	7.8
Lake 45	65	69	124	7.8
Lake 44	6	92	180	7.9
Lake 43	8	9	28	7.1
Lake 42	6	22	50	7.6
Lake 41	19	90	165	8.1
Lake 40	8	64	120	7.9
Lake 39	36	49	91	7.6
Lake 38	9	74	131	8.3
Lake 36	8	48	95	7.5
Lake 9	8	103	170	9.0
Lake 8	85	61	110	8.6
Pontoon	338	167	265	8.7
Lake 10 N.	16	92	160	8.1
Lake 10 S.	7	80	143	7.9
Madeline	98	72	210	7.8
Tom	15	61	128	7.1
Lake 7	30	107	210	7.5

TABLE 2. Spring total phosphorus, chlorophyll *a*, and summer mean Secchi transparency for the 49 study lakes.

Lake	[TP_{spr}] ($\mu g \cdot L^{-1}$)	[TP_{su}] ($\mu g \cdot L^{-1}$)	[Chl a_{su}] ($\mu g \cdot L^{-1}$)	[Chl a_{max}] ($\mu g \cdot L^{-1}$)	Secchi (m)
Lake 3	26.4	25.5	3.4	5.9	—
Lake 6	26.0	18.5	2.3	6.7	—
Lake 5	32.7	23.6	1.8	12.9	—
Fiddlers L. S.	28.3	48.0	13.1	18.2	0.7
Fiddlers L. N.	13.5	16.2	3.2	4.3	2.7
Fox	32.3	28.1	5.1	8.2	2.1
Long	16.6	14.4	2.6	4.0	3.2
Stock	33.1	24.1	7.2	11.2	2.2
Frame	20.6	22.2	6.9	11.0	—
Grace	35.3	19.3	5.7	14.1	2.8
Lake 1	29.7	22.8	3.3	10.0	—
Daigle	16.6	14.8	4.1	6.5	4.3
Finger	11.5	12.3	2.2	3.5	—
Shadow	19.8	22.4	4.2	5.1	—
X	21.0	20.1	2.1	2.7	—
Rater	34.0	47.0	4.1	6.8	3.5
Walsh	14.8	10.7	1.6	2.3	4.3
Vee	30.1	25.6	5.6	9.3	2.8
David	27.1	27.6	5.9	9.8	—
Gold	33.5	35.0	8.9	24.4	—
Gar	21.0	24.3	5.2	10.7	—
Trapper	30.3	28.0	1.8	5.6	—
Tibbit L. N.	16.8	10.5	2.0	2.6	4.8
Tibbit L. S.	11.1	7.6	1.9	2.5	5.0
Lake 50	33.4	46.2	11.3	16.0	0.6
Peninsula	6.4	8.9	2.3	2.8	3.3
Lake 49	15.4	18.7	4.1	5.0	1.7
Lake 48	16.9	18.0	3.2	4.2	—
Reid	7.9	7.5	1.5	2.2	4.4
Pickerel	18.5	21.8	8.1	9.8	1.6
Lake 47	26.3	24.5	5.5	13.2	—
Lake 46	19.8	24.1	7.3	11.7	3.0
Lake 45	21.8	22.4	6.4	9.5	2.3
Lake 44	31.0	19.9	4.4	8.4	1.5
Lake 43	11.1	7.7	3.2	4.9	2.9
Lake 42	30.4	39.3	8.6	11.7	1.0
Lake 41	37.7	35.4	11.5	19.2	0.9
Lake 40	20.9	20.8	3.4	6.5	2.9
Lake 39	21.1	16.1	3.2	5.3	3.9
Lake 38	51.4	63.0	16.9	20.4	0.6
Lake 36	16.4	13.1	2.2	4.7	—
Lake 9	34.7	48.2	12.7	17.1	0.8
Lake 8	43.2	52.8	26.8	42.7	0.5
Pontoon	13.4	11.1	2.6	3.4	3.3
Lake 10 N.	24.8	18.9	5.1	9.2	4.2
Lake 10 S.	19.6	17.4	4.1	7.5	4.3
Madeline	18.7	17.5	3.5	6.8	3.4
Tom	24.2	16.7	2.7	6.2	—
Lake 7	29.3	26.2	7.2	14.7	—

surface area and represented a range of spring total phosphorus concentration from 6 to 51 $\mu g \cdot L^{-1}$. Table 1 provides a brief description of each lake. Additional characteristics are given in Ostrofsky (1978a).

All lakes were sampled weekly from a single location in he geometric center of the lake, from spring ice melt in mid-May until September when most lakes were undergoing fall turnover. Samples for total phosphorus and chlorophyll *a* analyses were obtained using a weighted hose (1.25 cm i.d.) lowered to twice the Secchi disk depth. Aliquots of this integrated sample were placed in glass culture tubes with polypropylene caps for phosphorus and in litre jars for chlorophyll *a* analyses.

In the laboratory, phosphorus was determined using modifications of standard techniques. First, analysis was completed in the glass culture tubes to minimize the handling of the sample and reduce the chances of contamination. To each 50-mL sample, 0.4 g of potassium persulfate was added using a precalibrated Plexiglas scoop (modified from Menzel and Corwin 1965). Samples were then oxidized by autoclaving for 30 min. When cool, 5 mL of a sodium bisulfite – thiosulfate solution was added to eliminate arsenate interference (Seadler and Ostrofsky 1984, modified from Johnson 1971). After 30 min, 5 mL of a mixed reagent (Strickland and Parsons 1968)

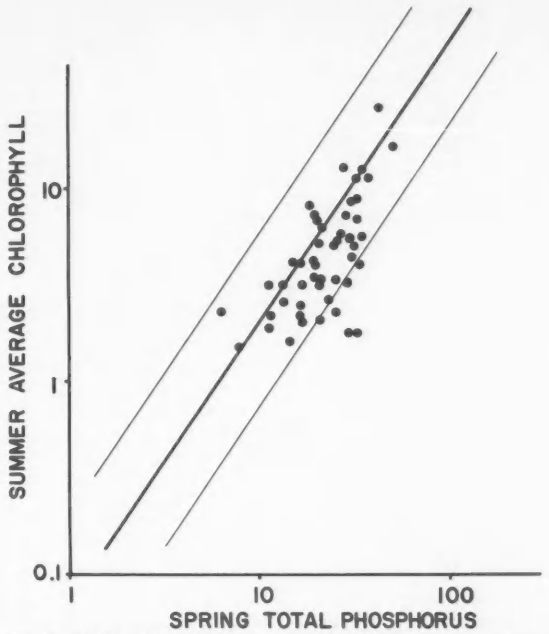

FIG. 1. Relationship between spring total phosphorus and mean summer chlorophyll concentration in 49 lakes near Yellowknife, N.W.T. Regression line and 95% confidence limits are from Dillon and Rigler (1974).

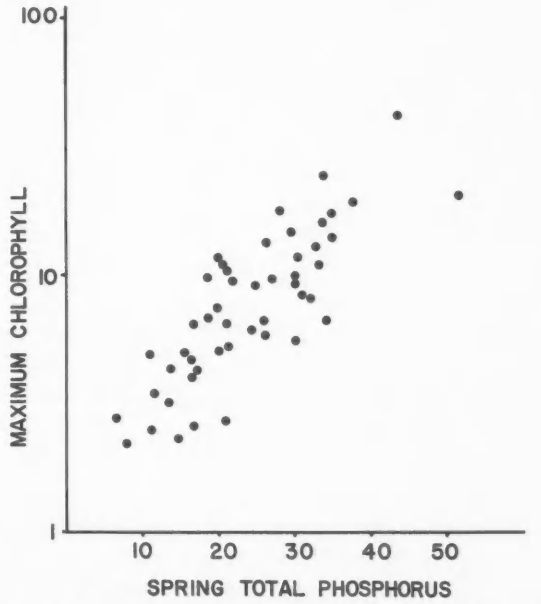

FIG. 2. Summer maximum chlorophyll concentration as a function of spring total phosphorus (exponential relationship).

was added. Color was allowed to develop for 30 min, and optical densities were read against a distilled water blank in a 10-cm cuvette at 885 nm. The use of a single container for sampling, oxidation, and color development was used by

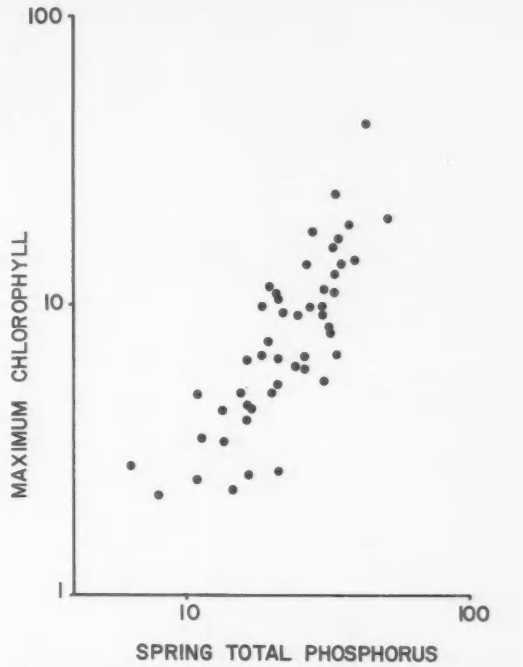

FIG. 3. Summer maximum chlorophyll concentration as a function of spring total phosphorus (power relationship).

Ostrofsky (1978a, 1978b), and the technique has proven to give estimates of phosphorus concentration with much reduced variances about the mean.

For the analysis of chlorophyll *a*, sample aliquots were filtered through glass fiber filters, and the filters were placed in 96% ethanol in the dark at 4°C for 24 h. After extraction, the slurry was refiltered through a second glass fiber filter, and the filtrate was collected and read against a 96% ethanol blank in a 10-cm cuvette at 750, 665, and 649 nm. Chlorophyll *a* concentrations were then calculated from the equations of Wintermans and DeMots (1965). Ethanol is more widely used in plant physiology research than in limnological research, but we have found that ethanolic extracts of phytoplankton chlorophyll are generally equal to acetone extracts, and when the phytoplankton population is composed of cyanobacteria or chlorophyta, the ethanolic extracts give a higher value for chlorophyll concentration (unpublished data).

Spring phosphorus refers to the concentration of total phosphorus during the spring overturn period. Summer chlorophyll refers to the mean concentration of chlorophyll *a* measured from the end of May to September. All empirical relationships were developed using least-squares linear regression analysis of the data, or standard transformations of the data.

Results and Discussion

Phosphorus, chlorophyll, and Secchi disk data are summarized in Table 2. The relationship between spring phosphorus concentration [TP_{spr}] and mean summer chlorophyll *a* concentration [Chl a_{su}] in the 49 Yellowknife lakes is shown in Fig. 1. Superimposed on these data are the regression line and

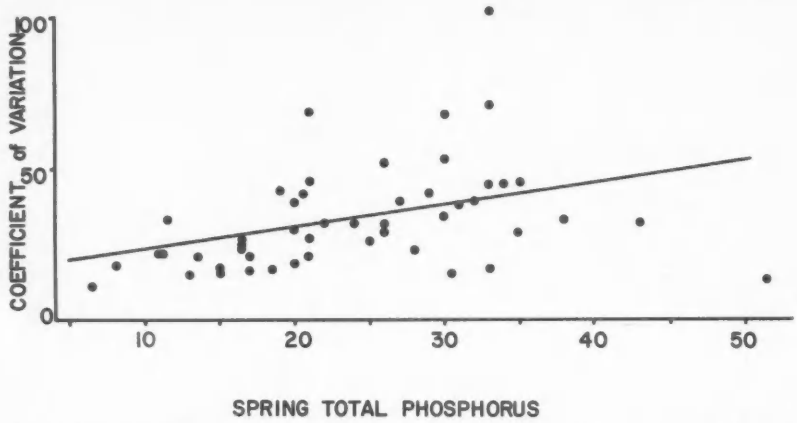

FIG. 4. Variability of phytoplankton populations, measured as the coefficient of variation of chlorophyll concentrations, as a function of spring total phosphorus.

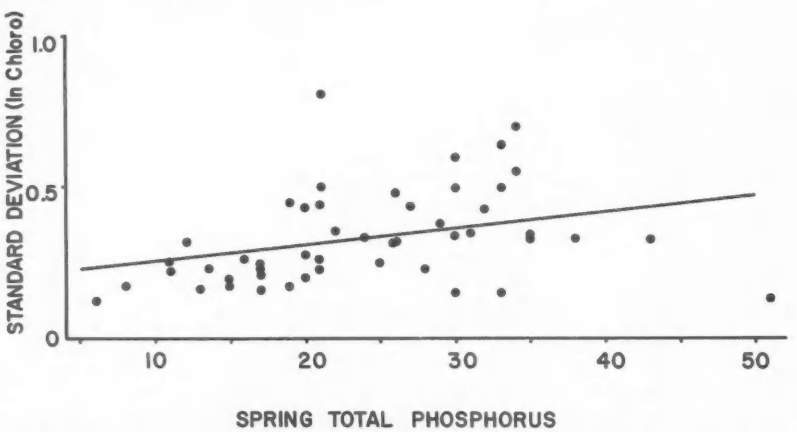

FIG. 5. Variability of phytoplankton populations, measured as the standard deviation of the logarithms of chlorophyll concentration, as a function of spring total phosphorus.

the 95% confidence limits for the Dillon and Rigler (1974) data set. The regression line for the Yellowknife data is

$$(1) \quad \log_{10}[\text{Chl } a_{su}] = -0.694 + 0.994 \log_{10}[\text{TP}_{spr}].$$

Spring TP explained less of the variation in summer chlorophyll ($r^2 = 0.42$) and the slope was significantly lower ($F_{1,91} = 6.19$, $p < 0.05$) in the Yellowknife data than in the Dillon–Rigler relationship. The Yellowknife results are, however, remarkably similar (slopes not significantly different) to those reported by Prepas and Trew (1983) for 29 lakes in Alberta ($F_{1,74} = 0.288$, $p > 0.05$) and Stockner and Shortreed (1985) for 50 lakes in British Columbia ($F_{1,95} = 1.08$, $p > 0.05$). Both Prepas and Trew (1983) and Stockner and Shortreed (1985), however, found that better predictions of mean summer chlorophyll *a* concentration could be made using mean summer total phosphorus [TP_{su}], and for data collected from 19 Yukon Territory lakes, Shortreed and Stockner (1986) found a relationship between only [TP_{su}] and [Chl a_{su}]. For the Yellowknife data, [TP_{su}] also explained more of the variation in [Chl a_{su}] ($r^2 = 0.63$) than did [TP_{spr}] although the correlation coefficients are not significantly different ($p > 0.05$). The regression line for the Yellowknife data is

$$(2) \quad \log_{10}[\text{Chl } a_{su}] = -0.728 + 1.035 \log_{10}[\text{TP}_{su}].$$

These results are again similar (slopes not significantly different) to the [TP_{su}]–[Chl a_{su}] relationships reported by Prepas and Trew (1983) for the Alberta lakes ($F_{1,79} = 0.499$, $p > 0.05$), Stockner and Shortreed (1985) for the British Columbia lakes ($F_{1,97} = 0.005$, $p > 0.05$), and Shortreed and Stockner (1986) for the Yukon Territory lakes ($F_{1,64} = 1.515$, $p > 0.05$).

In attempting to manage lakes, we often find that being able to predict summer average chlorophyll concentration is of limited use. We would like the ability to predict specific events, and in the context of lake management, one such specific event of interest is algal blooms. It would increase our ability to manage lakes better if we could predict both the magnitude and likelihood of these blooms which are indicated by intervals of high chlorophyll concentration. Figures 2 and 3

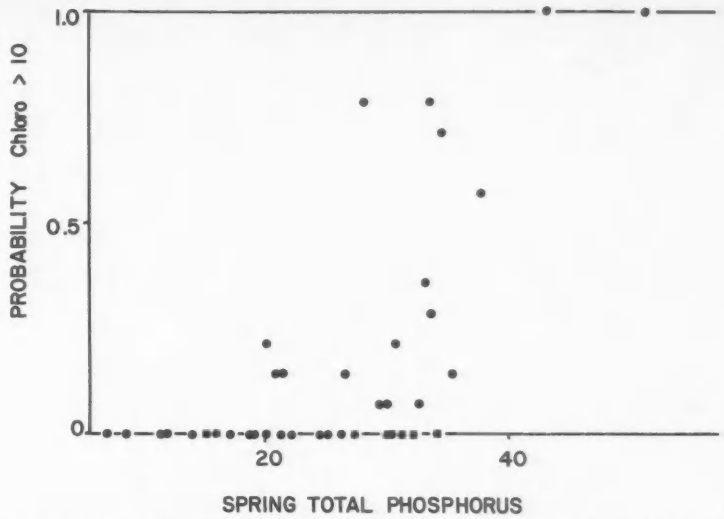

FIG. 6. Probability (relative frequency) of chlorophyll concentrations exceeding 10 $\mu g \cdot L^{-1}$ as a function of spring total phosphorus.

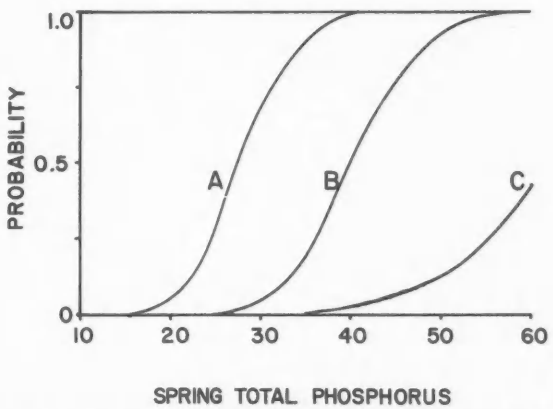

FIG. 7. Probability of chlorophyll concentration exceeding 5 $\mu g \cdot L^{-1}$ (curve A), 10 $\mu g \cdot L^{-1}$ (curve B), and 15 $\mu g \cdot L^{-1}$ (curve C) as functions of spring total phosphorus concentration. Curve C is truncated due to the absence of data beyond $[TP_{spr}] = 51\ \mu g \cdot L^{-1}$.

show the maximum observed summer chlorophyll *a* concentration as a function of $[TP_{spr}]$ for the 49 Yellowknife lakes. Both exponential and power functions fit the data equally well and we present both:

$$(3) \quad \log_{10} [\text{Chl } a_{max}] = 0.2664 + 0.0254 [TP_{spr}] \qquad r^2 = 0.67$$

$$(4) \quad \log_{10} [\text{Chl } a_{max}] = -0.8136 + 1.2576 \log_{10} [TP_{spr}] \qquad r^2 = 0.65.$$

There is no significant difference in the predictive power of these two models, although the second is in the same form as the $[TP_{spr}]$–$[\text{Chl } a_{su}]$ model, and as a result, comparisons between maximum and mean chlorophyll concentrations are more readily visualized.

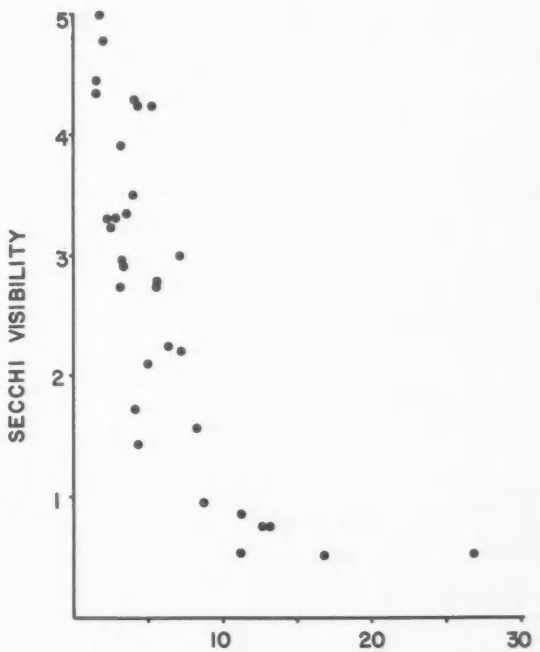

FIG. 8. Summer mean Secchi disk visibility as a function of mean summer chlorophyll.

The observation that the slope of the $[TP_{spr}]$–$[\text{Chl } a_{max}]$ regression is greater than the slope of the $[TP_{spr}]$–$[\text{Chl } a_{su}]$ regression indicates that with increasing total phosphorus concentration, the maximum chlorophyll concentration increases faster than does the average chlorophyll concentration. This

TABLE 3. Predicted chlorophyll data with 95% confidence limits in parentheses.

$[TP_{spr}]$ ($\mu g \cdot L^{-1}$)	[Chl a_{su}] ($\mu g \cdot L^{-1}$)	[Chl a_{max}] ($\mu g \cdot L^{-1}$)	$P([Chl\ a] > 5)$	$P([Chl\ a] > 10)$	$P([Chl\ a] > 15)$
5	1.00 (0.32–3.13)	1.16 (0.47–2.86)	0.00	0.00	0.00
10	2.00 (0.69–5.75)	2.78 (1.21–6.40)	0.00	0.00	0.00
20	3.97 (1.43–11.07)	6.65 (2.96–14.90)	0.06	0.00	0.00
30	5.95 (2.13–16.64)	11.07 (4.92–24.90)	0.73	0.05	0.01
40	7.91 (2.79–22.47)	15.89 (6.98–36.17)	0.99	0.49	0.03
50	9.88 (3.42–28.54)	21.04 (9.12–48.54)	1.00	0.94	0.14

observation is supported by Jones et al. (1979) who demonstrated that [Chl a_{max}] increased 1.7 times as fast as [Chl a_{su}] for about 50 North American and European lakes reported in the literature. The corresponding model derived using the Yellowknife lake data is

$$(5)\quad [\text{Chl } a_{max}] = 1.431[\text{Chl } a_{su}] + 1.471 \qquad r^2 = 0.85.$$

Equation (5) is quite similar to the model proposed by Jones et al. (1979) although they cannot be compared statistically in the absence of their raw data.

These models suggest that richer lakes have more variable algal populations with higher maxima and lower minima than nutrient-poor lakes, which is consistent with similar conclusions by Stoermer (1978) based on his observations of the St. Lawrence Great Lakes. Empirical relationships have been proposed which predict increasing variability in algal biomass as a function of increasing [Chl a_{su}]. Walker (1985) showed that the standard deviation of [Chl a] was related to [Chl a_{su}] in 148 Vermont lakes, and Walmsley (1984) proposed a similar model for 24 South African reservoirs. The corresponding models derived using the Yellowknife data are

$$(6)\quad \log_{10} s([\text{Chl } a]) = 1.038 \log_{10} [\text{Chl } a_{su}] - 0.556 \qquad r^2 = 0.85$$

$$(7)\quad s([\text{Chl } a) = 0.272[\text{Chl } a_{su}] + 0.278 \qquad r^2 = 0.64.$$

Equation (6) is quite similar to that proposed by Walker (1985) although statistical comparisons cannot be made. There is a significant difference ($F_{1,79} = 49.985$, $p < 0.05$) between equation (7) and the model proposed by Walmsley (1984) for the South African reservoirs.

To show this relationship more directly, we examined two measures which indicate variability in populations: coefficient of variation (Zar 1974) and the standard deviation of the logarithms of population size (Williamson 1972), and $[TP_{spr}]$ rather than [Chl a_{su}]. The data show a great deal of scatter (Fig. 4 and 5) but there is a significant relationship between trophic state as measured by $[TP_{spr}]$ and variability of the algal population as measured by [Chl a]:

$$(8)\quad \text{C.V.}[\text{Chl } a] = 17.2040 + 0.6647[TP_{spr}] \qquad r^2 = 0.12$$

$$(9)\quad s(\ln [\text{Chl } a]) = 0.2006 + 0.0054[TP_{spr}] \qquad r^2 = 0.11.$$

Both slopes are significant ($t = 2.58$ and 2.35, $p < 0.05$, respectively), although $[TP_{spr}]$ explains so little of the variability that [Chl a_{su}] remains the preferred predictor.

To estimate the likelihood of an algal bloom, we calculated the relative frequency with which a particular concentration of chlorophyll a was exceeded in the weekly observations from each lake. We interpreted this relative frequency as the probability that this chlorophyll concentration will be exceeded on any date, and graphed it as a function of $[TP_{spr}]$. In Fig. 6, we show the probability of [Chl a] > 10. The distribution of data points suggests a sigmoid relationship, so we used a logistic transformation (K. H. Reckhow, Duke University, pers. comm.) to find a best-fit regression line. The probability of exceeding a chlorophyll concentration of 10 $mg \cdot m^{-3}$ is given as

$$(10)\quad P([\text{Chl } a] > 10) = (1 + \exp(11.3252 - 0.2826[TP_{spr}]))^{-1} \qquad r^2 = 0.51.$$

In practice, a number of "critical" chlorophyll a concentrations may be considered and a family of curves generated (Fig. 7). For example

$$(11)\quad P([\text{Chl } a] > 5) = (1 + \exp(10.0818 - 0.3696[TP_{spr}]))^{-1} \qquad r^2 = 0.56$$

$$(12)\quad P([\text{Chl } a] > 15) = (1 + \exp(9.8196 - 0.1598[TP_{spr}]))^{-1} \qquad r^2 = 0.39.$$

The data do not support predictions beyond these critical concentrations. In practice, critical concentrations may be determined from the relationship between [Chl a] and Secchi disk visibility, and for the Yellowknife lakes, there is a dramatic decrease in water clarity between [Chl a] of 5 and 10 $\mu g \cdot L^{-1}$ (Fig. 8). The relationship between summer mean Secchi and [Chl a] for the Yellowknife lakes was

$$(13)\quad \log_{10} \text{Secchi} = 0.9369 - 0.8605 \log_{10} [\text{Chl } a_{su}] \qquad r^2 = 0.76$$

for 32 lakes where Secchi disk visibility was regularly measured, and for 446 individual observations

$$(14)\quad \log_{10} \text{Secchi} = 0.8665 - 0.7880 \log_{10} [\text{Chl } a] \qquad r^2 = 0.67.$$

Similar hyperbolic relationships between chlorophyll *a* and Secchi visibility have been proposed by Carlson (1977), Canfield and Hodgson (1983), and others.

Conclusions

Lakes in the vicinity of Yellowknife appear to behave in a similar manner as do lakes from more temperate regions. Mean summer chlorophyll *a* was highly correlated with spring total phosphorus, but the exact nature of this relationship was different from that developed by Dillon and Rigler (1974). The relationship was, however, similar to relationships presented for lakes in nearby Alberta, British Columbia, and the Yukon Territory. The reasons for the difference remain unexplained, although Shortreed and Stockner (1986) suggested that an abundance of large zoplankton grazers may be instrumental in preventing chlorophyll *a* concentrations from developing fully, and Stockner and Shortreed (1985) noted that in British Columbia monomictic lakes that are fast-flushing, the spring concentration of total phosphorus was a poor indicator of phosphorus available to phytoplankton during the summer growing season. These and other reasons may serve to explain the discrepancy in the response of chlorophyll to phosphorus in this region. Conversely, the observation that 40% of the data points used in the Dillon and Rigler phosphorus–chlorophyll model are from only two lakes (Washington and Minnetonka) suggests that this data set has lower variance than would be expected if each data point were a distinct lake.

The relationships between spring total phosphorus and maximum chlorophyll *a*, variability in chlorophyll concentrations, and relative frequency of exceeding critical chlorophyll concentrations developed here are similar to trends observed with data sets collected from other lake regions. We conclude that in this collection of subarctic lakes, chlorophyll responds to phosphorus in the same general manner as in temperate lakes. A summary of predictions and 95% confidence intervals is given in Table 3. While the confidence interval for any specific prediction is broad, these models represent a collection of useful tools for lake managers concerned with the impact of development in subarctic regions.

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Transfer of Organochlorine Residues from Blubber through the Circulatory System to Milk in the Lactating Grey Seal *Halichoerus grypus*

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Addison, R. F., and P. F. Brodie. 1987. Transfer of organochlorine residues from blubber through the circulatory system to milk in the lactating grey seal *Halichoerus grypus*. Can. J. Fish. Aquat. Sci. 44: 782–786.

Residues of the DDT group of insecticides and the polychlorinated biphenyls (PCBs) were measured in maternal blubber, milk, and blood or serum and in pup blubber and blood or serum of grey seals (*Halichoerus grypus*) from Sable Island, N.S. sampled in 1984 and 1985. DDT-group and PCB concentrations in blubber samples were lower in 1984 than in a sample of this population taken in the mid-1970's, but not lower than those in a sample taken in 1982. Concentrations in 1985 samples were slightly higher than those in the 1984 samples, for unknown reasons. On a lipid weight basis, residue concentrations in blood or serum were consistently about 50% of those in blubber lipid. The more highly chlorinated and therefore more lipid-soluble PCB components were transferred less efficiently from blubber to the circulatory system. Net transfer of residues from maternal blubber to maternal milk was about 70% efficient; this implies considerable degradation of circulatory lipid and resynthesis, probably to milk carbohydrates and other compounds. There was some selection against movement of especially the lower chlorinated PCB congeners from circulatory lipids into milk lipids, compared with transfer of the DDT group. Thus, a nonselective barrier exists to quantitative transfer of residues from blubber lipid to circulatory lipid, and a partially selective barrier exists against PCBs in the transfer of residues from circulatory to milk lipids; at least the latter barrier seems to involve lipid solubility.

Les auteurs ont quantifié les résidus d'insecticides du groupe DDT et des biphényles polychlorés (BPC) dans la graisse, le lait, le sang et le sérum de phoques gris (*Halichoerus grypus*) femelles et dans la graisse, le sang et le sérum de leurs nouveau-nés échantillonnés en 1984 et 1985 à l'île de Sable (N.-É.). Les concentrations de BPC et d'insecticides dérivés du DDT dans la graisse étaient plus faibles en 1984 que dans un échantillon de cette population recueilli vers le milieu des années 1970, mais elles n'étaient pas inférieures à celles observées dans un échantillon recueilli en 1982. Dans les échantillons prélevés en 1985, les concentrations étaient légèrement plus élevées que celles observées dans les échantillons de 1984, pour des raisons qui demeurent inconnues. En fonction du poids des lipides, les concentrations de résidus dans le sang ou le sérum s'élevaient constamment à environ 50 % de celles présentes dans les lipides de la graisse. Le transfert des composantes des BPC beaucoup plus chlorées et donc plus solubles dans les lipides s'est effectué moins efficacement de la graisse au système circulatoire. Le transfert net de résidus de la graisse au lait maternel était efficace à environ 70 %; ceci signifie une importante dégradation des lipides circulatoires et leur resynthèse, probablement en hydrates de carbone du lait et d'autres composantes. Il y a eu une certaine sélection contre le déplacement, surtout des congénères des BPC moins chlorés, à partir des lipides circulatoires vers les lipides du lait, par rapport au déplacement des résidus du DDT. Il existe donc un obstacle non sélectif au déplacement quantitatif de résidus à partir des lipides de la graisse vers les lipides du système circulatoire, ainsi qu'une barrière partiellement sélective des BPC pour ce qui du déplacement des résidus entre les lipides circulatoires et les lipides du lait. La solubilité des lipides semble être une composante de cette dernière barrière.

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Mammals may transfer organochlorine residues from maternal depot fat to offspring through lactation; the process has been demonstrated in humans (Jacobson et al. 1984; Siddiqui and Saxena 1985; Ando et al. 1985) and in the ferret (Bleavins et al. 1984). In marine mammals the process may be particularly important, since relatively large amounts of depot lipid are mobilised for production of a lipid-rich milk, usually over a short period. Thus, although marine mammals may transfer organochlorine residues from mother to pup during foetal development (Anas and Wilson 1970), probably much more is transferred during lactation (Addison and Brodie 1977). Using our previous data from the grey seal, *Halichoerus grypus* (Addison and Brodie 1977) and taking pup length (l) to be 100 cm, girth (g) to be 70 cm, blubber thickness at birth (bt) to be 10 mm, blubber density (d) to be 0.9, and ΣDDT concentration in blubber (concn.) to be 2 $\mu g \cdot g^{-1}$, we can calculate the amount of ΣDDT in a newborn pup to be

$$l \times g \times \text{bt} \times d \times \text{concn.} = 1.3 \times 10^{-2} \text{ g (approx.)}.$$

Thus, 13 mg of ΣDDT would have been laid down during foetal development. This figure is almost certainly an overestimate, since at birth the grey seal pup has practically no measurable blubber (P. F. Brodie, unpubl. data). In contrast, the amount of ΣDDT available from milk during a 15-d lactation is about 340 mg (Addison and Brodie 1977). That is, by the time the pup is weaned, at least 95% of its residue burden would have come from milk, even allowing for some loss of assimila-

TABLE 1. Ages, blubber thickness (mm), blubber lipid content (% wet wt.), and organochlorine residue concentrations ($\mu g \cdot g$ lipid wt.$^{-1}$) in seal mothers and pups (*Halichoerus grypus*) sampled on Sable Island, N.S., in 1984 and 1985. Data presented as means ± SD; data in the same row followed by different letters differ significantly by *t*-test ($P<0.05$).

	1984 (n = 6)	1985 (n = 9)
Mothers		
Age (yr)	17.5 ± 7.1 a	10.6 ± 2.7 b
Blubber thickness	47.5 ± 6.0	50.1 ± 7.7
Blubber lipid	92.0 ± 5.2	93.5 ± 2.3
p,p'-DDE	1.75 ± 0.59 a	2.64 ± 1.09 b
p,p'-DDT	0.64 ± 0.31 a	1.07 ± 0.53 b
ΣDDT	2.39 ± 0.83 a	3.71 ± 1.41 b
PCB	16.2 ± 6.80 a	30.3 ± 17.0 b
Pups		
Age (d)	3 ± 2	4 ± 1
Blubber thickness	20.0 ± 9.2	24.7 ± 9.5
Blubber lipid	68.7 ± 28.4	86.7 ± 10.5
p,p'-DDE	1.44 ± 0.59	1.20 ± 0.46
p,p'-DDT	0.36 ± 0.17	0.28 ± 0.14
ΣDDT	1.81 ± 0.74	1.48 ± 0.57
PCB	10.4 ± 6.2	8.8 ± 4.1

tion of residues during digestion.

Lactation is therefore a very important factor in the distribution of organochlorines in marine mammals, and deserves close study. Previously, we suggested that some "barrier" existed to the quantitative transport of residues from maternal blubber into milk and that this barrier was also selective to certain compounds such as polychlorinated biphenyls (PCBs) (Addison and Brodie 1977). We undertook the work described here to define how or where this barrier occurs, and to explain the mechanism of its selectivity.

Experimental

Isolated mother–pup pairs of grey seals were killed on Sable Island, N.S., in January 1984 (six pairs) and in January 1985 (nine pairs). Lengths, girths, and blubber thicknesses were recorded and samples (minimum 15 g where possible) of mid-dorsal maternal blubber, blood, and milk (obtained by dissecting mammary tissue) and of pup mid-dorsal blubber and blood were taken. Samples were frozen for some weeks at −10°C pending analysis, except for the 1985 blood samples which were heparinised and held at 37°C for a couple of hours before being centrifuged to prepare serum, which was then frozen at −10°C.

Samples were analysed for lipids and for organochlorine residues generally as described by Addison and Brodie (1977) but using capillary column GLC (e.g. Addison et al. 1986). Standards run included National Research Council of Canada chlorobiphenyl congener standard mixes CB-1 to CB-4.

Adult seals were aged by counting growth layers on longitudinal tooth sections (Laws 1962); pups were aged by condition and appearance of the pelage (Addison and Brodie 1984).

Results

Organochlorine residue concentrations in blubber samples taken in 1984 and 1985 are shown in Table 1. Residues found

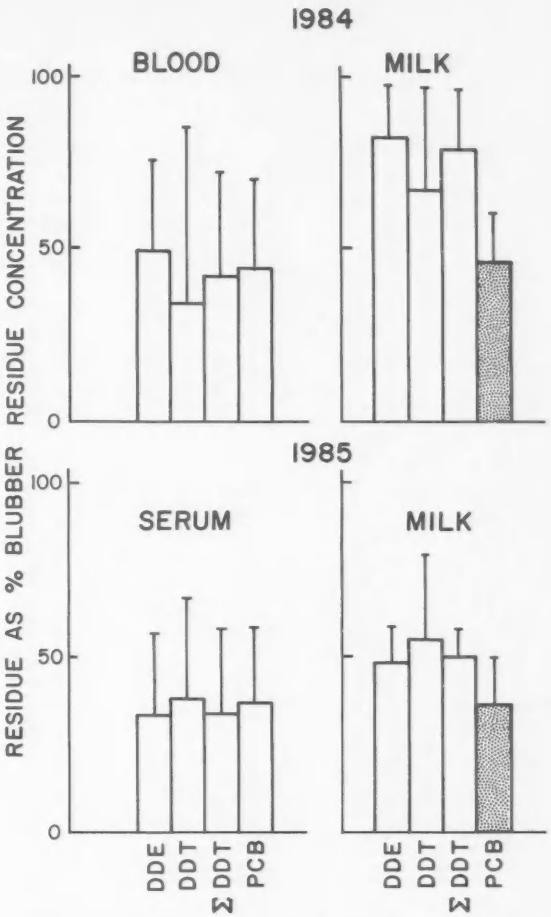

FIG. 1. Organochlorine residue groups in blood or serum lipid and milk lipid from grey seals (*Halichoerus grypus*) from Sable Island, N.S., sampled in 1984 and 1985. Data are presented as means ± SD of concentrations expressed as a percentage of concentrations in blubber lipid. The shaded bars differ significantly ($P < 0.05$) from other data from the same tissue.

included *p,p'*-DDT, *p,p'*-DDE, and PCBs; *p,p'*-DDD may have been present, but was obscured by a PCB component. Organochlorine concentrations in maternal blubber sampled in 1985 were significantly higher ($P < 0.05$) than in samples taken in 1984. The 1985 samples were from significantly younger seals than those taken in 1984, but residue concentrations in reproducing females are independent of age (Addison and Smith 1974). However, both 1984 and 1985 concentrations were lower than those measured in this population in 1974 and 1976, but not lower than those measured in 1982 (Addison and Brodie 1977; Addison et al. 1984). Pups generally had lower concentrations than did adults. Only about 25% of the PCB components could be positively identified on the basis of retention time by comparison with available standard mixtures; however, these components were fairly evenly distributed throughout the chromatogram and implied a mixture ranging from tri- to hepta-chlorobiphenyls. Compounds identified as IUPAC Nos. 138, 153, and 180 (Ballschmiter and

TABLE 2. Lipid content (% wet wt.) and organochlorine residue concentrations (μg·g lipid wt.$^{-1}$) in blood or serum from grey seal (*Halichoerus grypus*) mothers and pups sampled on Sable Island, N.S., in 1984 and 1985. Data presented as means ± SD.

	1984 (n = 6)			1985 (n = 9)		
Sample	Maternal blood	Milk	Pup blood	Maternal serum	Milk	Pup serum[c]
Lipid content	0.77±0.27	36.2 ±10.8[a]	1.07±0.32	1.00±0.18	38.9 ±14.5[b]	1.38±0.36
p,p'-DDE	0.76±0.16	1.42± 0.43	0.92±0.45	0.82±0.54	1.22± 0.35	0.95±0.20
p,p'-DDT	0.08±0.13	0.38± 0.09	0.04±0.04	0.32±0.21	0.58± 0.38	0.42±0.09
ΣDDT	0.84±0.25	1.80± 0.49	0.96±0.48	1.14±0.75	1.81± 0.71	1.38±0.29
PCB	6.08±1.28	7.48± 3.14	6.24±2.92	10.21±6.02	10.12± 3.29	11.84±3.19

[a] n = 5.
[b] n = 6.
[c] n = 8.

Zell 1980) predominated and together accounted for 60% of the identifiable components.

The distribution of residues in blood (or serum) and in milk is shown in Table 2. Lipid contents were similar to those reported by Nelson (1970) for harp and elephant seals. Pup blood or serum contained consistently higher lipid than did the corresponding maternal samples, possibly reflecting the pup's constant feeding on milk. The same organochlorine residues were found as in the blubber samples but the ratios of the components differed, blood and milk generally containing less p,p'-DDT, and milk containing appreciably lower amounts of PCBs. This apparent selectivity in the transfer of some organochlorine components from blubber to milk is illustrated in Fig. 1, in which the relative concentrations of the main residues have been expressed as a percentage of blubber concentrations. It then becomes clear that (1) movement of residues from blubber into blood or serum is about 50% efficient, and shows no selectivity, and (2) net transfer of residues from blubber to milk is from about 50–70% efficient, at least for the DDT group, but is appreciably less for the PCBs. This implies that there is considerable selectivity in the transfer of residues from the circulatory system into milk. Figure 2 presents similar data for specific PCB congeners: unlike the DDT group, there appears to be some selectivity in movement of PCB components from maternal blubber into serum, as well as some selectivity in the net transfer of residues from blubber to milk.

Discussion

Blubber thickness and lipid contents of the samples are similar to those reported previously for this species (Ackman and Jangaard 1965; Addison and Brodie 1977). The major differences between the present data and those obtained previously from this population are in organochlorine residue concentrations: maternal blubber residue concentrations seem to have declined between the mid-1970s and 1982 (Addison et al. 1984) but not from 1982 to 1985. This conclusion is supported by the fact that the fraction of ΣDDT represented by p,p'-DDE — an indication of the "age" of the DDT group (Aguilar 1984) — was significantly higher in 1982 than in 1974 or 1976, but did not differ between 1982 and 1984 or 1985 ($P > 0.05$). Thus, the decline in total DDT-group concentrations which occurred between the mid-1970s and the early 1980s appears to have slowed somewhat. PCB levels were anomalous: although there was no significant difference in mean PCB concentrations in blubber between 1976, 1982, and 1984, levels in 1985 were significantly higher ($P < 0.05$). We cannot explain this, but note that it is consistent with our previous conclusion that PCB concentrations in the eastern

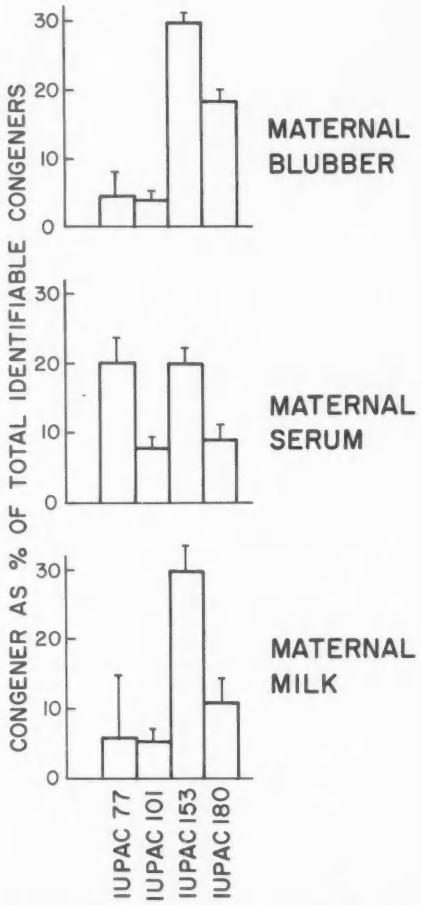

FIG. 2. Distribution of PCB congeners IUPAC Nos. 77, 101, 153, and 180 in maternal blubber, serum, and milk lipid from grey seals (*Halichoerus grypus*) sampled in 1985 from Sable Island, N.S. Data are presented as the percentages of total identifiable PCB congeners represented by individual components (means ± SD) in tissue lipid.

Canadian seals may be declining more slowly than those of the DDT group (Addison et al. 1984).

As we found previously, the transfer of organochlorine residues from maternal blubber to milk was incomplete when calculated on a lipid weight basis. Mean transfer of ΣDDT was 79 ± 18% (mean ± SD, $n = 6$) in 1984 and 49.2 ± 9.2% ($n = 9$) in 1985. Previously, we estimated that about 60% of blubber lipid ΣDDT was transferred to milk lipid (Addison and Brodie 1977). Also, as we noted previously, PCBs were transferred less efficiently than the DDT group: 48 ± 13% in 1984 and 37 ± 13% in 1985. These differences between the PCB and DDT groups were significant ($P < 0.01$ in 1984; $P < 0.05$ in 1985). Figure 1 provides some insight into the process of organochlorine transfer from blubber to milk. All major groups of residues are mobilised to about the same extent (although there are differences within the PCB group, discussed below). The slight differences between 1984 and 1985 blubber to blood transfer may reflect the presence of erythrocytes in the 1984 whole blood samples, which may affect lipid extractability or which may have adsorbed some organochlorine residues. As the lactating grey seal does not feed, the bulk of her circulatory lipids must be derived from blubber, rather than from chylomicra arising from lipid sources in her diet. Therefore, the reduced organochlorine concentrations in serum or blood indicate a partial barrier to quantitative mobilisation from blubber. The nature of this barrier is not clear.

Another barrier appears to exist in the transfer of residues from the circulatory system to milk. On a lipid weight basis, organochlorine concentrations were higher in milk than in blood or serum, which implies more than 100% transfer of circulatory organochlorines. This is not impossible, if we regard the circulatory system as being closed but with a certain steady-state concentration of organochlorines which are continuously "extracted" into milk lipids which are then secreted in batches. The only assumption to be made is that organochlorines can move from circulatory lipid into milk lipid against a concentration gradient. However, a more likely explanation may be that the differences between blood and milk organochlorine concentrations reflect incomplete transfer of lipid from circulatory system to milk, but a more efficient transfer of organochlorines. Milk lipids in general are only partly derived from circulatory lipid: from 20 to 80% of milk lipid is synthesised in the mammary gland, mainly from carbohydrate sources (e.g. Baumann and Davis 1974; Patton and Jensen 1976). Thus, the different organochlorine concentrations in circulatory and milk lipids could result from degradation and resynthesis of circulatory lipid in the mammary gland, with some possible loss of associated organochlorines. Additional milk lipids may be synthesised in the mammary gland from carbohydrates. That considerable metabolic activity does occur in the grey seal mammary gland is implied by the appreciable differences in fatty acid distributions in blubber and milk (Ackman and Jangaard 1965). The lower concentrations of PCBs in circulatory than in milk lipids (Fig. 1) also indicate a partial barrier to organochlorine transfer. To summarise, then, it appears that a barrier exists to the quantitative transfer of organochlorine residues from maternal blubber to milk, both at the stage of mobilisation of blubber lipid to circulatory lipid (this barrier probably being nonselective) and at the circulation – mammary gland stage, this barrier being distinctly selective against PCBs.

The distribution pattern of PCB congeners in blubber, circulatory lipid, and milk (Fig.2) suggests some factors which may govern these transfers. Consider first the transfer of PCB congeners from blubber to serum. In the series IUPAC Nos. 77, 101, 153, and 180 (a tetra-, penta-, hexa- and heptachlorobiphenyl, respectively), the relative concentrations (serum lipid/blubber lipid) are 5.0, 2.1, 0.7, and 0.5. That is, the higher the molecular weight (or the extent of chlorination) the less efficient is the transfer from blubber lipid to serum lipid. Almost the reverse trend is seen in the transfer of congeners from serum lipid to milk: ratios (milk lipid/serum lipid) are, for the same series, 0.3, 0.7, 1.5, and 1.2. It is not clear whether this trend reflects differences in molecular weight (or size, or shape) or differences in lipid solubility, although these factors are all interrelated. No direct measurements of lipid solubility have been made on these compounds, but Matthews et al. (1984) have used hexane/acetonitrile partition coefficients as a measure of lipid solubility and have concluded that within the series IUPAC Nos. 77, 101, and 153, lipid solubility increases. (No data are available for IUPAC No.180.) Therefore, the trend towards increasing accumulation of chlorobiphenyls in blubber or milk lipids, as compared with serum lipid, parallels the trend towards increasing lipid solubility. The serum lipid pool in the fasting mother seal is probably composed mainly of the more polar lipids and lipoproteins (cf. Nelson 1970) in which chlorobiphenyls may be less soluble than in nonpolar depot lipids. Further evidence in support of this comes from the observations of Matthews et al. (1984) that chlorobiphenyls partitioned less readily into the lipoproteins of blood taken from fasted animals than into those from animals which had been fed fat, presumably because the total circulatory lipid pool in the former was smaller.

The reason that we see no difference in *p,p'*-DDT and *p,p'*-DDE transfer from blubber to milk is presumably because their molecular weights, 354 and 318, respectively, and their octanol–water partition coefficients (an indirect measure of lipid solubility) are similar (McDuffie 1981; Chiou et al. 1977), implying that their lipid solubilities should also be similar.

Measurement of organochlorines transferred from milk through the digestive system of the pup and into pup blood or serum and into blubber did not lead to any clear conclusions. Data varied somewhat between 1984 and 1985 samples, but in general, there appeared to be no consistent selectivity towards any particular residue. However, interpretation of the data was complicated by the fact that circulatory system residues depend (presumably) on both dietary sources and current blubber residue composition. "Digestibility" of residues, as indicated by pup blood lipid concentrations as a fraction of milk lipid concentrations, was around 80–90%. Blubber lipid concentrations were, in general, not significantly different from blood or serum lipid concentrations. The generally lower concentrations in pup blubber than in maternal blubber arise probably from two sources: (1) nonquantitative transfer of residues from maternal blubber into milk and (2) some loss during digestion and assimilation of milk.

In conclusion, the transfer of organochlorine residues from blubber to circulatory lipid in the lactating seal may be explained by a simple partition model based on organochlorine lipid solubility. Transfer of organochlorines between circulatory lipid and milk lipid is more complex, due to the metabolism of circulatory lipid at the mammary gland and the resynthesis of milk lipid from circulatory lipid and carbohydrate sources. This process is associated with some selectivity against PCBs.

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Genetic Changes in Pink Salmon (*Oncorhynchus gorbuscha*) Following Their Introduction Into the Great Lakes

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Pink salmon (*Oncorhynchus gorbuscha*) previously thought to require salt water for completion of their life cycle, have been firmly established in the Great Lakes following an accidental introduction into Lake Superior. We compare allelic frequencies at 27 protein-coding loci from collections of pink salmon from the Great Lakes with those from the anadromous population (Lakelse River, British Columbia) from which they were derived. Although the allelic frequencies in the Great Lakes collections are consistent with a single introduction, the frequencies observed in these collections differ substantially from those of the Lakelse River population. Alleles of *G3p-1* and *Ck-1*, rarely observed (frequency < 0.005) in British Columbia population, are present in Great Lakes pink salmon at frequencies between 0.06 and 0.27. Smaller changes were observed at 11 other loci; 14 loci were monomorphic in all collections. Loss of variability in the Great Lakes was reflected by a decrease in average number of alleles per locus. Selection for physiologically tolerant phenotypes may have been necessary to establish this unique, self-perpetuating, freshwater population. The biochemical genetic changes we observed, however, can be adequately explained by genetic drift resulting from bottlenecks occurring at the first and at subsequent generations. Any decreases in survival resulting from freshwater intolerance would have exacerbated the bottlenecks. This adaptively distinct lineage produced by the ecological change coupled with the bottlenecks may be a major step toward speciation.

On croyait que le saumon rose (*Oncorhynchus gorbuscha*) avait besoin d'eau salée pour compléter son cycle vital, mais il s'est établi avec succès dans les Grands Lacs suite à son introduction accidentelle dans le lac Supérieur. Les auteurs comparent les fréquences alléliques à 27 locus codeurs de protéine chez des échantillons de saumon rose recueillis das les Grands Lacs avec celles de la population anadrome mère (rivière Lakelse, Colombie-Britannique). Quoique les fréquences alléliques dans les échantillons des Grands Lacs indiquent une introduction unique, ces fréquences sont très différentes de celles observées dans la population de la rivière Lakelse. Les allèles de *G3p-1* et *Ck-1*, rarement observés (fréquence $< 0{,}005$) chez la population de la Colombie-Britannique, sont présents chez le saumon rose des Grands Lacs à des fréquences allant de 0,06 à 0,27. De plus petites variations ont été observées à 11 autres locus; de plus, 14 locus étaient monomorphes dans tous les échantillons. La perte de variabilité des allèles chez le saumon rose des Grands Lacs s'est traduite par une baisse du nombre moyen d'allèles par locus. Une sélection pour des phénotypes physiologiquement tolérants peut avoir été nécessaire à l'établissement de cette population unique de saumon dulçaquicole indépendante. Les variations génétiques biochimiques que nous avons observées peuvent être facilement expliquées par les fluctuations génétiques entraînées par des étranglements au cours de la première génération et des générations ultérieures. Une baisse de la survie suite à l'intolérance aux eaux douces aurait aggravé les étranglements. Cette lignée distincte à cause de son adaptation, produite par des variations écologiques alliées à des étranglements, peut être un important pas vers la spéciation.

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Pink salmon (*Oncorhynchus gorbuscha*) were introduced into the Great Lakes in 1956 when approximately 21 000 fry intended for transplant to a tributary of Hudson Bay were inadvertently released in Lake Superior (Schumacher and Eddy 1960; Nunan 1967). This was an exotic introduction (Krueger et al. 1981) because the Great Lakes lie outside the natural geographical and environmental range of pink salmon. The Great Lakes pink salmon comprise the only known self-perpetuating freshwater populations (MacKay 1963; Emery 1981), notable because many attempts either to transplant pink salmon into a lacustrine environment or to raise pink salmon of marine parentage in freshwater without making dietary adjustments to compensate for the lower salinity have had little success (Bakshtansky 1962; Hurley and Woodall 1968; Senn and Buckley 1978).

In their natural range in the northern Pacific Ocean and Bering Sea, pink salmon have a rigid 2-yr anadromous life cycle to which exceptions are exceedingly rare (Gilbert 1913; Davidson 1934; Rounsefell 1958; Anas 1959; Bilton and Ricker 1965; Bakshtansky 1962; Turner and Bilton 1968). The temporal separation resulting from the life cycle of this species has actually produced two genetically distinct lines, each spawning either in even or in odd years (Aspinwall 1973, 1974a, 1974b; Johnson 1979; McGregor 1982; A. J. Gharrett and A. J. McGregor, in prep.). In contrast, in the Great Lakes, exceptions to the 2-yr life cycle are evidenced by the emergence of both even- and odd-year populations from the single 1956 transplant (Kwain 1978: Kwain and Chappel 1978; Wagner and Stauffer 1980; Kwain and Lawrie 1981; Wagner and Stauffer 1982). In fact, 3-yr-old pink salmon are not uncommon in the Great Lakes, and mixtures of mature 1-, 2-, and 3-yr old fish have been reported in some spawning populations

TABLE 1. Protein-coding loci, their Enzyme Commission (EC) numbers (International Union of Biochemistry 1984) and designations (May 1980), and the tissues and buffers in which they were resolved. The peptidase loci are designated according to their substrate specificity. *Pep(Pp-2)* is peptidase D activity and *Pep(Lgg-1)* is peptidase B activity (Frick 1983). Buffers are I (Ridgway et al. 1970), II (Markert and Faulhaber 1965), III (Clayton and Tretiak 1972), and IV, a pH 7.0 tris(hydroxymethyl)aminomethane buffer described by Shaw and Prasad (1970).

Enzyme	EC No.	Locus designation	Tissue	Buffer
Aconitate hydratase	4.2.1.3	*Aco-3*	Heart, muscle	IV
		Aco-4	Heart, muscle	IV
Adenosine deaminase	3.5.4.4	*Ada-1*	Muscle	III
		Ada-2	Muscle	III
Aspartate aminotransferase	2.6.1.1	*Aat-3*	Eye	I
Creatine kinase	2.7.3.2	*Ck-1*	Muscle	I
		Ck-2	Muscle	I
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi-1,2*	Muscle	I
		Gpi-3	Muscle	I
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3p-1*	Muscle	II, III
Lactate dehydrogenase	1.1.1.27	*Ldh-1*	Muscle	I
		Ldh-2	Muscle	I
		Ldh-4	Liver	I
		Ldh-5	Eye	I
Malic enzyme	1.1.1.40	*Me-1*	Muscle	III
Mannose-6-phosphate isomerase	5.3.1.8	*Mpi*	Heart, eye	II
Malate dehydrogenase	1.1.1.37	*Mdh-1,2*	Liver, muscle	III
		Mdh-3,4	Muscle	II, III
Peptidases	3.4.11/13			
Leucyl-glycyl-glycine activity		*Pep(Lgg-1)*	Muscle	I
Phenylalanyl-proline activity		*Pep(Pp-1)*	Muscle, heart	II
		Pep(Pp-2)	Muscle	II
Glycyl-leucine activity		*Pep(Gl-1)*	Muscle	II
Phosphoglucomutase	5.4.2.2	*Pgm-2*	Muscle	I, II
Phosphogluconate dehydrogenase	1.1.1.44	*Pgd*	Muscle	III, IV

(Kwain and Chappel 1978; Wagner and Stauffer 1980; Kwain and Kerr 1984).

We have compared the biochemical genetic compositions of several collections of pink salmon sampled from the Great Lakes with that of pink salmon taken from the odd-year from Lakelse River, British Columbia, the source of the transplant. Here we report genetic differences between the Great Lakes pink salmon and the parental stock.

Materials and Methods

Collections of tissues for electrophoretic analysis were taken from adult pink salmon on or near spawning grounds. In 1985, tissues were taken from pink salmon returning to spawn in Lakelse River, a tributary to the Skeena River in northern British Columbia. Six collections of adults were also taken from the Great Lakes as follows. Lake Superior: Cross River, Minnesota, in 1980 and Pancake River, Ontario in 1981; Lake Huron: Albany Creek, Michigan, and Carp River, Michigan, in 1981 and Black Mallard River, Michigan in 1985; Lake Michigan: Black River, Michigan, a stream geographically near the Lake Huron streams, in 1981.

Tissue extracts were electrophoretically analyzed as described by Utter et al. (1974). Biochemical genetic loci (designations and Enzyme Commission numbers), tissues in which the loci are expressed, and buffers used to resolve the activities are given in Table 1.

Departures from Hardy–Weinberg equilibrium expectations were examined with chi-square goodness-of-fit tests with a continuity correction of 0.25 (Emigh 1980). Homogeneity of allelic frequencies among populations was examined using log-likelihood ratio analysis (*G*-test, Sokal and Rohlf 1981). For these tests, pooling of genotypic or allelic frequencies, respectively, was done to eliminate expected classes of less than four.

Estimation of average heterozygosities and their sampling variances (Nei and Roychoudhury 1974; Nei 1978) and genetic distances corrected for sample sizes (Nei 1972, 1978) were made using a FORTRAN program obtained from Nei and modified. Clustering of these distances was done by unweighted pair-group arithmetic average (UPGMA, Sneath and Sokal 1973). Genetic distances to a node were presumed to be greater than zero if heterogeneity (*G*-test) existed between the two branches joined at the node. In the analyses described above, the variability observed at *Mdh-3,4*, co-migrating, duplicated loci (isoloci, Allendorf and Thorgaard 1984) was treated as if all the variability appeared at one locus and the other was monomorphic. We have found that our treatment of isoloci tends to give conservative estimates of genetic distances.

Results

Fourteen of 27 loci examined were monomorphic and identical in all collections. These were *Aco-3*, *Ada-1*, *Ck-2*, *Ldh-1*, *-2*, *-4*, and *-5*, *Mpi*, *Pgd*, *Pep(Pp-1)*, and isoloci *Gpi-1,2* and *Mdh-1,2*.

For the polymorphic loci (Table 2), it should first be noted that the Mendelian nature of inheritance has been demonstrated

in pink salmon for *G3p-1*, *Pep(Pp-2)*, *Aat-3*, *Pgm-1*, *Ada-2*, *Me-1*, *Mdh-3,4* and *Aco-4* (Johnson 1979; McGregor 1982; A. J. Gharrett and A. J. McGregor, in prep.) and in two closely related species, *O. kisutch* and *Salmo gairdneri*, for *Ck-1* (Utter et al. 1979). These loci are the ones at which most of the variability was observed. No deviations from Hardy–Weinberg equilibrium expectations were observed ($P > 0.05$) for *Pep(Pp-2)* and *Aat-3*, the only loci sufficiently variable and with large enough sample sizes to be tested; however, only large deviations would be detected in the collections as small as those from the Great Lakes.

Examination of allelic frequencies at the polymorphic loci shows that the transplants differed strikingly from the donor population (Table 2). (i) No variability was observed at seven of the loci displaying variability in the Lakelse River collection (*Aco-4*, *Pep (Lgg-1)*, *Pep (Gl-1)*, *Gpi-3*, *Pgm-1*, and *Mdh-3,4*). At each of these loci, the frequency of the common allele in the Lakelse collection was greater than or equal to 0.90. (ii) The more common variant allele (*200*, frequency = 0.075) at the glycerol-3-phosphate dehydrogenase locus (*G3p-1*) was absent in Great Lakes pink salmon, but an allele (*120*) rare in the donor population was present at frequencies ranging from 0.09 to 0.18. (iii) A creatine kinase (*Ck-1*) electromorph (*80*) not observed in the donor sample was present in Great Lakes collections at frequencies ranging from 0.06 to 0.27. While absent in the sample of the donor population ($N = 100$), this allele is found at very low frequencies in some nearby southeastern Alaskan populations (McGregor 1982; A. J. McGregor and A. J. Gharrett, in prep.). If the alleles were present in the Lakelse population at similar frequencies, omission of these alleles from the sample would not be unlikely. Indeed no individuals homozygous for the *Ck-1* (*80*) allele were observed.

In the Great Lakes collections, variability was observed at four other loci which were also polymorphic in the Lakelse population. Frequencies of all three alleles at the *Pep (Pp-2)* locus reflect the general similarity of the Great Lakes collections and their divergence from the donor populations. At the other three loci (*Aat-3*, *Ada-2*, and *Me-1*), the allelic frequencies of the Great Lakes and Lakelse collections were similar.

The genetic compositions of the Great Lakes collections displayed little heterogeneity ($P = 0.04$), as described by log-likelihood ratio analysis of allelic frequencies (Sokal and Rohlf 1981), and were more similar to each than they were collectively to the donor population ($P \ll 0.001$). Phenetic relationships also demonstrate the similarity of Great Lakes collections and their divergence from the donor population (Fig. 1). Heterozygosities (Nei 1978) of 27 biochemical genetic loci did not change, 0.060 ± 0.023 (SE) for the donor and an average of 0.062 with SE's ranging between 0.029 and 0.032 for the transplants. The average number of alleles per locus, however, decreased from 1.6 in the donor to 1.3 in the Great Lakes. The genetic distance (Nei 1978) between the donor and transplated populations is comparable with that observed between collections of even-year pink salmon taken from the extremes of the North American range; however, the genetic distance is less than that observed between even- and odd-year lines of anadromous pink salmon (McGregor 1982; A. J. Gharrett and A. J. McGregor, in prep.).

Discussion

The similarity among the collections from the Great Lakes, both between even- and odd-year collections and among geographically distinct locations, is consistent with a single introduction. These data suggest that some divergence may have occurred among newly founded colonies in the Great Lakes, but the small number of collections precludes a systematic examination of the genetic relations among pink salmon in the Great Lakes.

The genetic differences between the transplanted pink salmon and the donor population occurred in less than 26 yr, 12–13 generations for pink salmon. Stability of biochemical genetic polymorphisms has been observed in southeastern Alaskan pink salmon populations for seven generations (McGregor 1982; A. J. Gharrett and A. J. McGregor, in prep.). In the Great Lakes, no substantial changes occurred at the *G3p-1* and *Ck-1* loci between 1980 and 1985 (Table 2), and heterogeneity among collections displayed by variable loci is small ($P = 0.041$). Since the donors were taken from a large population, we conclude that the divergence occurred during the first 26 yr after the transplant.

Two processes could be responsible for the divergence between the Great Lakes and donor populations, natural selection and/or random genetic drift. The potential for considerable genetic diversity existed in the approximately 21 000 transplanted fry, which was a subsample of more than 750 000 intended for Hudson Bay and produced from more than 500 dams and approximately the same number of sires. Selection for individuals that could tolerate the physiological demands of an exclusively freshwater environment must have occurred in the establishment in the Great Lakes of this species generally requiring salt water during some life stages. If natural selection was the important factor producing the biochemical genetic changes we observed, one could hypothesize that the alleles at the *G3p-1* and *Ck-1* loci which are rare in the donor population but more abundant in the Great Lakes pink salmon are involved with or closely linked to discrete loci important in freshwater adaptation. It is interesting, however, that the pink salmon fry introduced into Hudson Bay, although presumably better preadapted physiologically to the marine environment, failed to establish pink salmon populations (Nunan 1967). Presumably, other elements of the Hudson Bay environment differ more drastically from the natural environment than does that of the Great Lakes.

The alternative hypothesis, random genetic drift, results from errors inherent in small samples. The transplanted fry which survived to reproduce were the first sample. Survival of fry to maturity in Pacific Ocean populations is variable but of the order of 2%; most of the mortality is attributable to capricious factors such as predation and food availability (Pritchard 1948; Hunter 1959). Returns of fry transplanted from one Pacific Ocean drainage to another are often considerably lower than those returning to native streams (Pritchard 1938; Ricker 1972; Wickett 1958; Bams 1976). Diminished homing precision is one factor that may reduce returns of transplanted fry as much as 10-fold (Bams 1976). Homing precision is especially important in a colonizing population because inaccurate homing drastically reduces the ability of mature adults to find a mate in an appropriate environment.

The rapid spread of pink salmon throughout the Great Lakes after 1969 and the sporadic use of some spawning habitat (W. Taylor, Department of Fisheries and Wildlife, Michigan State University, MSU, East Lansing, MI 48824, pers. comm.) is consistent with a diminished homing ability. Using estimations of survival (approximately 2%) and homing ability of pink salmon transplanted within their natural range, an estimate

TABLE 2. Biochemical genetic variation in collections of pink salmon from the Great Lakes and Lakelse River, B.C. Allelic frequencies and by their electrophoretic mobility relative to the most common allele (mobility 100) observed in both the Great Lakes and Pacific Ocean; the

	G3p-1					*Ck-1*			*Pep(Pp-2)*				*Aat-3*			*Ada-2*		
Site and year of collection	*N*	100	200	175	120	*N*	100	80	*N*	100	109	93	*N*	100	85	*N*	100	87
British Columbia																		
Lakelse River (1985)	100	0.915	0.075	0.005	0.005	100	1.00	0.00	100	0.65	0.25	0.10	100	0.79	0.21	99	0.93	0.07
Lake Superior tributaries																		
Cross River (1980)	33	0.91	0.00	0.00	0.09	33	0.94	0.06	33	0.42	0.32	0.26	32	0.73	0.27	33	0.89	0.11
Pancake River (1981)	54	0.83	0.00	0.00	0.17	54	0.92	0.08	54	0.45	0.32	0.23	51	0.78	0.22	51	0.98	0.02
Lake Huron tributaries																		
Carp River (1981)	26	0.83	0.00	0.00	0.17	26	0.73	0.27	26	0.48	0.40	0.12	21	0.79	0.21	26	0.96	0.04
Albany Creek (1981)	17	0.82	0.00	0.00	0.18	17	0.88	0.12	17	0.35	0.41	0.24	17	0.65	0.35	17	0.97	0.03
Black Mallard River (1985)	24	0.90	0.00	0.00	0.10	24	0.85	0.15	—	—	—	—	—	—	—	—	—	—
Lake Michigan tributary																		
Black River (1981)	44	0.83	0.00	0.170	0.000	44	0.82	0.18	44	0.43	0.34	0.23	35	0.87	0.13	43	0.99	0.01

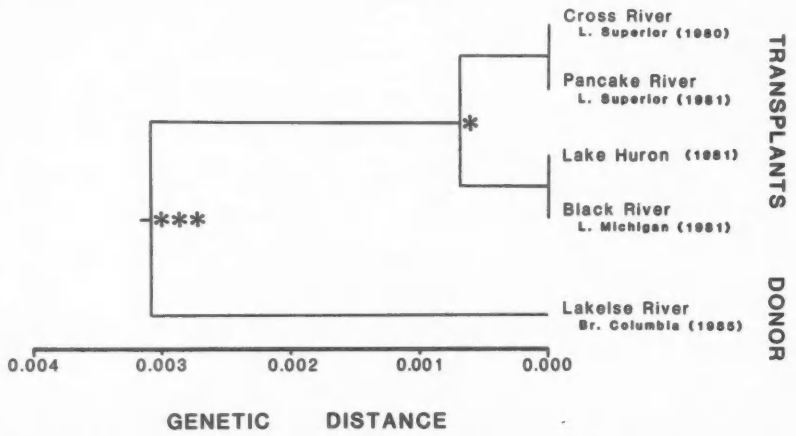

FIG. 1. Phenetic relationships among the Great Lakes and Skeena River, B.C., collections. Nei's (1978) standard genetic distances were estimated from 27 biochemical genetic loci and clustered using UPGMA (Sneath and Sokal 1973). Isoloci (Allendorf and Thorgaard 1984) were treated as if the variability existed at one of the loci. Data from two Lake Huron collections taken in 1981, which showed no heterogeneity ($P = 0.12$) in log-likelihood analysis and which were geographically in close proximity, were pooled because of their small sample sizes. Asterisks denote junctions for which heterogeneity (log-likelihood ratio) exists between the two branches ($*P < 0.05$; $***P < 0.001$).

of the number of fish surviving to breed in the first generation is on the order of $0.02 \times 0.1 \times 21\,000$ or 42 individuals. Inability of some individuals to adapt physiologically to the freshwater environment would further decrease the size of the first generation. For example, only one adult was recovered from transplants of approximately 10 000 pink salmon fry into two closed lakes in Siberia (Bakshtansky 1962). The sizes of the first generations of pink salmon in the Great Lakes are unknown; however, they probably were small. Because the introduction was accidental, efforts to monitor the success of pink salmon in the Great Lakes occurred only after two fish were discovered in 1959 (Schumacher and Eddy 1960), two generations after the introduction. Even in 1961, after three generations, very few fish were found (Schumacher and Hale 1962), and not until after the sixth generation were large numbers and rapid dispersal observed (Lawrie and Rahrer 1972). By 1979, pink salmon had been observed spawning in tributaries of all five Great Lakes (Emery 1981; Kwain and Lawrie 1981; Wagner and Stauffer 1982).

Observations regarding the nature of genetic change at other loci are consistent with the genetic drift hypothesis. Loci for which only modest levels of polymorphism were observed in the donor populations (frequency of common allele > 0.95) became fixed for their common alleles. Loci with more polymorphism (frequency of common allele < 0.90) retained their polymorphism, although in some cases with altered frequencies.

The *G3p-1* and *Ck-1* loci did display substantial changes between donor and derived lines; however, even these changes are not unexpected in small samples. For example, if the frequency of the rare *Ck-1* allele in the donor population was comparable with those in populations of nearby southeastern

collection sizes (N) for polymorphic biochemical genetic loci are presented. Loci are described in Table 1. Alleles at a locus are designated sum of frequencies of all alleles at a locus is unity.

Me-1				*Mdh-3,4*				*Pgm-1*			*Aco-4*			*Pep(Gl-1)*			*Gpi-3*			*Pep(Lgg-1)*		
N	100	130	70	*N*	100	130	70	*N*	100	150	*N*	100	115	*N*	100	98	*N*	100	98	*N*	100	125
100	0.94	0.07	0.00	100	0.90	0.08	0.02	100	0.95	0.05	89	0.98	0.02	100	0.995	0.005	96	0.98	0.02	100	0.995	0.005
33	1.00	0.00	0.00	33	1.00	0.00	0.00	33	1.00	0.00	33	1.00	0.00	33	1.00	0.00	33	1.00	0.00	33	1.00	0.00
54	0.93	0.07	0.00	54	1.00	0.00	0.00	54	1.00	0.00	54	1.00	0.00	54	1.00	0.00	54	1.00	0.00	54	1.00	0.00
26	0.94	0.02	0.04	26	1.00	0.00	0.00	26	1.00	0.00	26	1.00	0.00	26	1.00	0.00	26	1.00	0.00	26	1.00	0.00
17	0.97	0.03	0.00	17	1.00	0.00	0.00	17	1.00	0 .00	17	1.00	0.00	17	1.00	0.00	17	1.00	0.00	17	1.00	0.00
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
44	0.90	0.09	0.01	44	1.00	0.00	0.00	44	1.00	0.00	44	1.00	0.00	44	1.00	0.00	44	1.00	0.00	44	1.00	0.00

Alaska (0.0057 for $N = 959$) (McGregor 1982; A. J. Gharrett and A. J. McGregor, in prep.) and the frequency of the *G3p-1* allele (*120*) observed in the donor is accurate (0.005), over 3% of samples of 20 individuals would have at least one of each of these alleles. Over 10% of samples of 40 individuals would have at least one of each allele.

Selection for pink salmon physiologically more tolerant to freshwater occurred during establishment of pink salmon in the freshwater of the Great Lakes, an environment that was previously considered hostile to them. Osmoregulation is a complicated process determined by numerous loci. It is improbable that the few simple Mendelian loci we observed were of overwhelming importance in the adaptation of pink salmon to the Great Lakes; and although substantial differences between the donor and transplants were observed in the allelic compositions of biochemical genetic loci, these differences are adequately explained by a small founding population.

The adaptation to a presumably hostile environment coupled with the breakdown of a formerly rigid year-class structure is consistent with the notion that the pink salmon of the Great Lakes is a lineage adaptively distinct from the parent population. This adaptation includes a bottleneck of at least one — and probably more — generation. Bottlenecks coupled with abrupt and strong adaptive changes are components of transilience modes of speciation in contrast with divergence modes where spatial isolation occurs across a continuum (Templeton 1980). Relocation experiments to the original environment and physiological comparisons are suggested to test to what extent a major step has been made towards speciation.

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Comparing Imprecise Abundance Indices with a Symmetric Model

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Richards, L. J. 1987. Comparing imprecise abundance indices with a symmetric model. Can. J. Fish. Aquat. Sci. 44: 793–802.

Richards and Schnute (1986. Can. J. Fish. Aquat. Sci. 43: 1214–1227) presented an errors-in-variables model for testing the assumption that catch per unit effort (CPUE) is an index of stock abundance. Here, I generalize the model and extend its application to any pair of abundance indices for which the ratio of variances in the two indices can be estimated. I used the model to relate density indices from two years, CPUE values from two years, and the two CPUE–density combinations. Density estimates of nearshore reef fishes from the strait of Georgia, British Columbia, were obtained by SCUBA, and CPUE estimates were obtained by angling at the same sites. Among-site differences in both CPUE and density tended to be consistent between years. For quillback rockfish (*Sebastes maliger*), the relationship between CPUE and density was one of strict proportionality, as found by Richards and Schute over a different depth range.

Richards et Schnute (1986. Can. J. Fish. Aquat. Sci. 43: 1214–1227) ont présenté un modèle d'erreur des variables pour la vérification de l'hypothèse que les prises par unité d'effort (PUE) sont un indice de l'abondance d'un stock. Dans la présente étude, l'auteur généralise le modèle et étend son application à toute paire d'indices de l'abondance pour lesquels on peut estimer le rapport des variances. Il utilise le modèle pour établir une relation entre les indices de la densité pendant deux ans, les PUE pendant deux ans et les deux combinaisons PUE–densité. Les estimations de la densité des poissons peuplant les récifs côtiers du détroit de Géorgie (Colombie-Britannique) ont été obtenues par plongée autonome tandis que les estimations des PUE ont été obtenues par pêche à la ligne aux mêmes endroits. Les différences entre les sites pour ce qui est des PUE et de la densité avaient tendance à être uniformes d'une année à l'autre. Dans le cas du sébaste à dos épineux (*Sebastes maliger*), la relation entre les PUE et la densité était nettement proportionnelle comme l'avaient mentionné Richards et Schnute pour un différent écart de profondeur.

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Catch per unit effort (CPUE) of a fish stock is often used as an index of stock abundance (Gulland 1964; Paloheimo and Dickie 1964; Bannerot and Austin 1983). Recently, Richards and Schnute (1986) tested the assumption that CPUE and abundance are proportionally related. They developed an errors-in-variables model that allowed for several forms for the relationship between CPUE and density, and a statistical testing procedure to decide among them. They made visual observations of reef-fish density from a submersible in the Strait of Georgia, British Columbia, and compared these density estimates with CPUE estimates obtained by angling at the same sites. For quillback rockfish (*Sebastes maliger*), the dominant species, they found that the relationship was strictly proportional. However, the shape of the relationship between CPUE and the density index was poorly determined for other species combinations. This was because the model allowed for error in both the density and CPUE measurements. A low CPUE at high density could be accounted for by error in the density measurements.

Here, I generalize the Richards and Schnute (1986) model and extend its application to any pair of abundance indices for which the ratio of variances in the two indices can be estimated. I select a more extensive data set with two years of CPUE and density data. I determine the shape of the relationship between CPUE and density at a site for different combinations of reef fish species and then ask whether or not the shape is consistent between years. I also examine the shape of the between-year relationships for CPUE and for density measurements at the sites. The density data were obtained from SCUBA surveys of reef fishes conducted in 1984 and 1985 in the Strait of Georgia, British Columbia, and the CPUE data were obtained by angling at the same sites.

Experimental Methods

The study was conducted in the northeast section of the Strait of Georgia (Fig. 1), at 16 sites in 1984, and at 17 sites in 1985. There were minor differences in sites between years because of access problems at some sites. Angling was conducted in August 1984 from the M/V *KETA*, a 12-m research seiner, and in June and July 1985 from a 7-m aluminum skiff. We used standard recreational angling gear with 12-cm frozen bait herring. A data record included the combined effort of two or three anglers, each with one line, and represented a minimum of approximately 0.5 h of total fishing time. Records were deleted from the analysis if less than 10 min of fishing was completed before sea conditions changed. We fished over a range of depths, although I report here only on data from

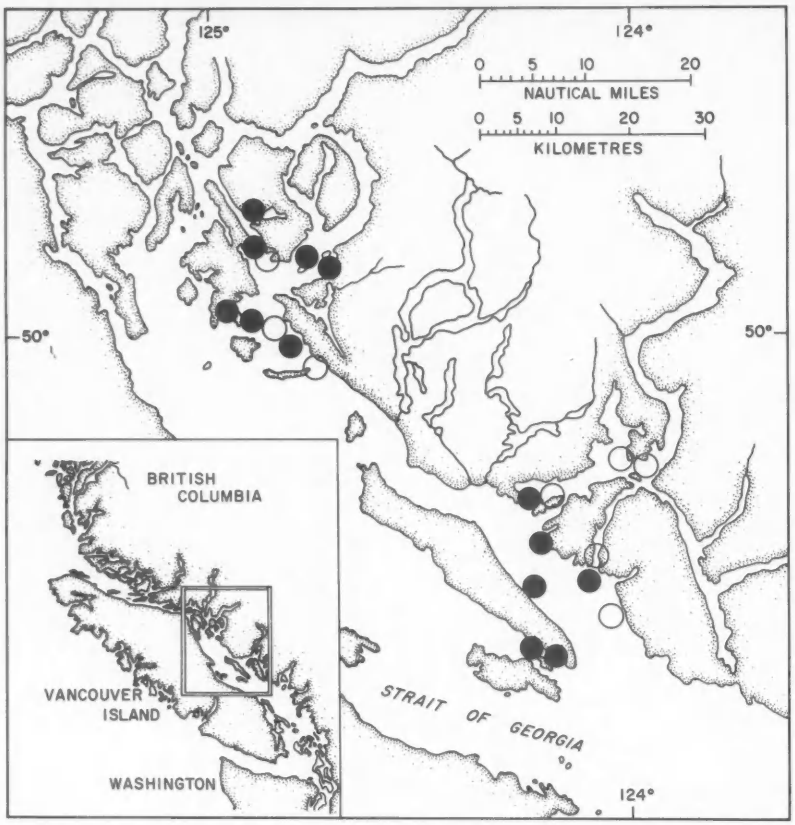

FIG. 1. Locations of SCUBA and angling sites in the Strait of Georgia, British Columbia. Open circles indicate locations of additional angling sites.

5–40 m. We occasionally encountered large concentrations of spiny dogfish (*Squalus acanthias*). I excluded records if spiny dogfish accounted for 50% or more of the catch. Raw data and further methodological details are presented in Richards et al. (1985a) and Richards and Cass (1985).

SCUBA surveys were conducted between September and November of 1984 and 1985 at 13 of the sites for which angling data were available (12 sites in each year). Divers counted all rockfish, lingcod, greenling, and most other fish species that were visible from 50-m horizontal transects. A total of 120 transects were completed each year, with 10 transect locations selected at random at each site. In addition to fish counts, fish size (fork length) was visually estimated for most species. These size estimates were accurate to within approximately 5% (Richards et al. 1986). Small and cryptic species such as gobies, flatfishes, and sculpins were excluded from the surveys because of the difficulty in obtaining representative counts. Visibility was estimated as the horizontal distance at which bars on a black and brown patterned disc (to represent a quillback rockfish) could no longer be distinguished by the diver. To determine the relationship between visibility and transect width, the lateral displacement of each fish from the transect line was estimated during 55 of the transects in 1985. Different divers participated in the survey in the two years. Further methodological details are presented in Richards et al. (1985b, 1986).

Analytical Methods

Symmetric Model

Because of the structure of the CPUE and density data, I required a model with greater flexibility than the model of Richards and Schnute (1986). Following their notation, let U represent the CPUE (fish per line per hour) and D represent an index of density (fish per square metre). The Richards and Schnute (1986) model consists of the two conditions

$$(1a)\quad U = (p + qD)/(1 + rD) \text{ if } D > -p/q$$

$$(1b)\quad U = 0 \text{ if } D \leq -p/q$$

where p, q, and r are parameters constrained so that

$$(2a)\quad qr \geq 0$$

$$(2b)\quad q \geq pr.$$

This model has the feature that when $r \neq 0$, the curve (1a) approaches the value $U = q/r$ asymptotically as D becomes large. For the CPUE and density data presented here, it was also necessary to allow for a curve that could approach the value $D = -1/r$ asymptotically as U becomes large. This

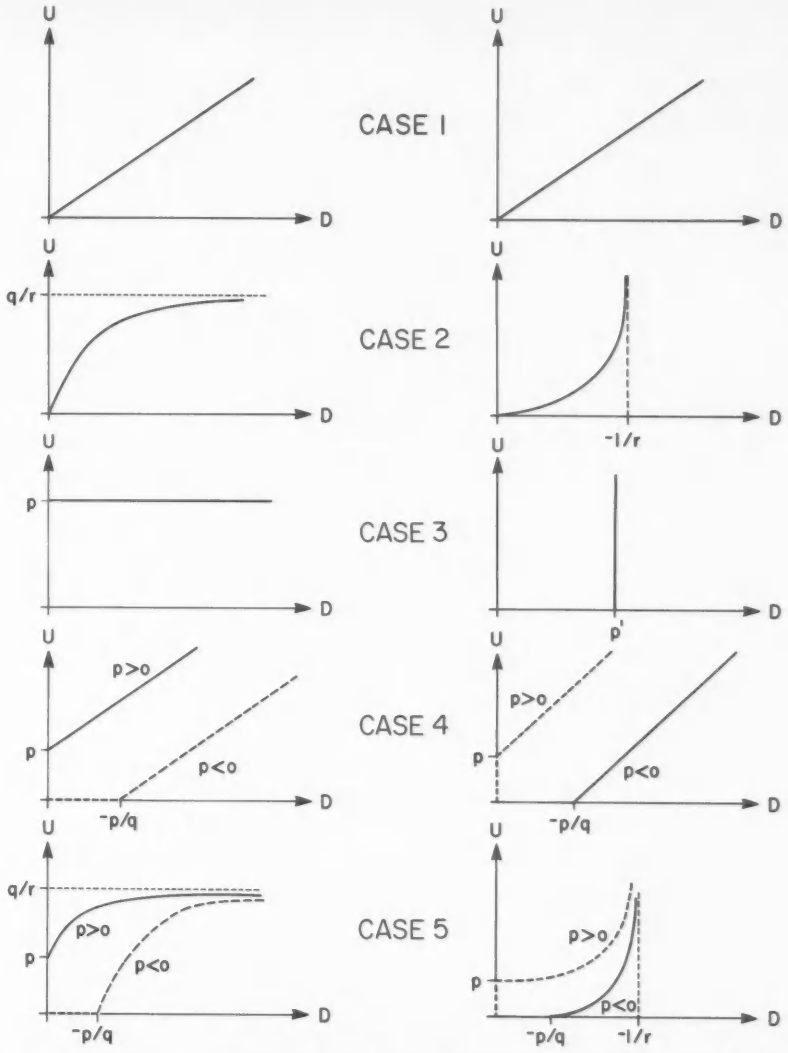

FIG. 2. Typical curve forms for five cases of the relationship between CPUE (U, fish·line^{-1}·h^{-1}) and the density index (D, fish·m^{-2}) for model 1 (left-hand panels) and the corresponding cases for model 2 (right-hand panels). Parameter values are indicated where curves cross the axes. Multiple solutions are shown if the curve form depends on parameter values.

second possibility is not allowed by the constrained model (1)−(2) above, as it defines U for all possible values of D. However, a model with a D-asymptote can be obtained by reversing the roles of D and U in (1)−(2). This gives

$$(3a)\quad D = (p' + q'U)/(1 + r'U) \text{ if } U > -p'/q'$$

$$(3b)\quad D = 0 \qquad \text{if } U \leq -p'/q'$$

where p', q', and r' are parameters constrained so that

$$(4a)\quad q'r' \geq 0$$

$$(4b)\quad q' \geq p'r'.$$

If $q \neq 0$, it is possible to relate equation (3a) to (1a), ignoring all constraints. The formal equivalence of parameters (p, q, r) and (p', q', r') is as follows:

$$(5a)\quad p = -p'/q'$$

$$(5b)\quad q = 1/q'$$

$$(5c)\quad r = -r'/q'.$$

Equations (5) must be interpreted cautiously, as models (1)−(2) and (3)−(4) are not equivalent when constraints are involved. The utility of (5) is that it allows (p', q', r') to be represented in the same dimensional units as (p, q, r) for easier comparison. The dimensional significance of (p, q, r) is discussed by Richards and Schnute (1986).

Model 1 (1)−(2) and model 2 (3)−(4) each have five cases that are biologically interesting (Fig. 2). For case 1, the rela-

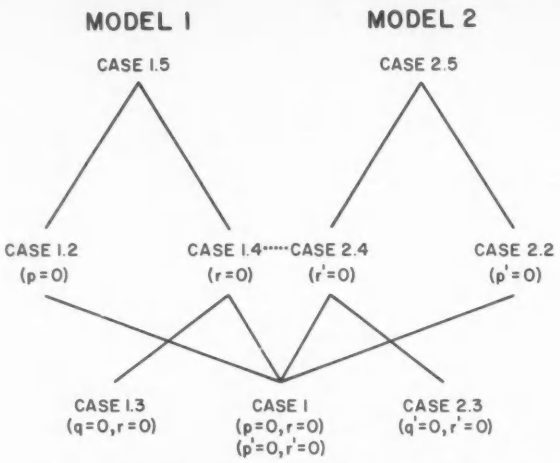

FIG. 3. Hierarchy of case selection for models 1 and 2.

tionship between U and D is one of strict proportionality. Case 2 is similar to case 1 at low values of U and D, but asymptotic at high values. For case 3, the value of either U or D is constant. For case 4, the relationship is linear but does not pass through the origin. Case 5 is asymptotic (as case 2), but does not pass through the origin (as case 4). Note that the models are symmetrical and that neither U nor D is preferred as an abundance index in the analysis.

Where it is necessary to distinguish cases of the two models in the description below, I will designate a case number as model number − decimal − case number. For case 1 ($p = 0$, $r = 0$) or ($p' = 0$, $r' = 0$), the two models are identical with $q' = 1/q$ (5b). For cases 1.4 ($r = 0$) and 2.4 ($r' = 0$), the models are identical with the transformation (5a)−(5b) in the range where both conditions (1a) and (3a) apply. Cases 2, 3, and 5 are not comparable between models as the curves approach the U-asymptote for model 1 and the D-asymptote for model 2.

Statistical Error and Parameter Estimation

To consider the statistical error associated with the application of model 1, let d_i ($i = 1, \ldots, k$) represent the observed density index at the ith site and let D_i be the corresponding true, but unobserved, density index. D_i represents a theoretical density that would be obtained from an infinite number of transects at site i. Similarly, let u_i be the observed CPUE at the ith site with a corresponding true value U_i. Then, model 1 consists of the following assumptions:

(6a) $U_i = (p + qD_i)/(1 + rD_i)$ if $D_i > -p/q$

(6b) $U_i = 0$ if $D_i \leq -p/q$

(7) $d_i^{\beta} = D_i^{\beta} + \sigma f_i \delta_i$

(8) $u_i^{\gamma} = U_i^{\gamma} + \tau g_i \epsilon_i$

where β and γ are power transformations to compensate for heteroscedastic variance, assumed known, and δ_i and ϵ_i are independent normal random variables with mean 0 and variance 1. The quantity σf_i in (7) is the standard deviation of d_i^{β} where σ is a fixed parameter and the quantities f_i ($i = 1, \ldots, k$) are known weighting factors. Similarly, the quantity τg_i in (8) is a standard deviation and g_i is a weighting factor that compensates for heteroscedastic variance. Independent estimates of β, γ, f_i, and g_i are possible because there were replicate measures of CPUE and density at a site. Details of computing β, γ, f_i, and g_i and the site mean observations are given in the data analysis section below.

Assumptions (7)−(8) were modified from Richards and Schnute (1986) to more generally accommodate the structure of CPUE and density data. Recent articles have reported that the distribution of CPUE, for example, is not normal (Bannerot and Austin 1983; Quinn 1985), and Quinn (1985) used the power transformation to model CPUE of Pacific halibut. As a method for parameter estimation, the power transformation results in biased estimates. An approximate bias correction can be obtained by the delta method (Seber 1982, p. 7). The intent here is hypothesis testing rather than parameter estimation, however, and this point is not considered further.

The likelihood function L associated with model 1 is

$$L = (\beta\gamma)^k (2\pi\sigma\tau)^{-k} \left[\prod_{i=1}^{k} d_i^{(\beta-1)} u_i^{(\gamma-1)} (f_i g_i)^{-1} \right] \times \exp\left[-\frac{1}{2} \sum_{i=1}^{k} (\delta_i^2 + \epsilon_i^2) \right].$$

This is the likelihood function from Richards and Schnute (1986) except for the product

$$(\beta\gamma)^k \prod_{i=1}^{k} (d_i^{(\beta-1)} u_i^{(\gamma-1)})$$

that results from the variable transformations in (7)−(8). If I define

$$(9) \quad A = k \log(\lambda\sigma^2) + 1/2(\sigma)^{-2} \sum_{i=1}^{k} [(d_i^{\beta} - D_i^{\beta})/f_i]^2 + 1/2(\lambda\sigma)^{-2} \sum_{i=1}^{k} \{[u_i^{\gamma} - [(p + qD_i) \div (1 + rD_i)]^{\gamma}]/g_i\}^2$$

where

$$(10) \quad \lambda = \tau/\sigma,$$

then A is equal to the negative log likelihood ($-\log L$), except

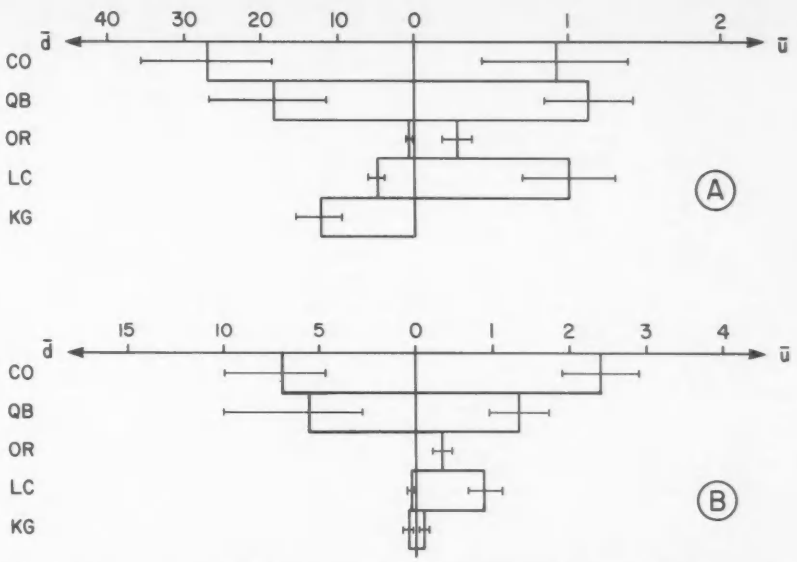

FIG. 4. Overall mean density ($\bar{d}$, fish·(100 m^2)$^{-1}$) with one standard error and overall mean CPUE ($\bar{u}$, fish·line^{-1}·h^{-1}) with one standard error for the major species that were observed or caught during (A) 1984 and (B) 1985. CO, copper rockfish; QB, quillback rockfish; OR, other rockfish; LC, lingcod; KG, kelp greenling.

for a constant independent of parameters.

By analogy, model 2 consists of assumptions (7)–(8) and

$$(11a)\quad D_i = (p' + q'U_i)/(1 + r'U_i) \quad \text{if } U_i > -p'/q'$$

$$(11b)\quad D_i = 0 \quad \text{if } U_i \leq -p'/q'.$$

For this model, the function

$$(12)\quad A' = k \log(\lambda\sigma^2) + 1/2(\sigma)^{-2} \sum_{i=1}^{k} \{[d_i^\beta - [(p' + q'U_i) \div (1 + r'U_i)]^\beta]/f_i\}^2 + 1/2(\lambda\sigma)^{-2} \sum_{i=1}^{k} [(u_i^\gamma - U_i^\gamma)/g_i]^2$$

is similarly equal to the negative log likelihood except for a constant.

The function A depends on the unknown $4 + k$ parameters p, q, r, and σ and the true densities $D_1, D_2, \ldots, D_k$. Similarly, the function A' depends on the unknown $4 + k$ parameters p', q', r', and σ and the true CPUE values $U_1, U_2, \ldots, U_k$. Parameters for models 1 and 2 can be estimated as a constrained minimization on A or A', respectively. A maximum likelihood estimate for σ is described in Richards and Schnute (1986).

Hypothesis Testing

Richards and Schnute (1986) described a methodology for choosing the most appropriate of the five possible cases of model 1, by suitable restrictions of the parameters p, q, and r. They defined the statistic

$$(13)\quad B = 2(A_0 - A_1)$$

where A_0 is the minimum value of A under a null hypothesis with h_0 parameters and A_1 is an alternative hypothesis with $h_1 > h_0$ parameters. By the likelihood ratio test, B is approximately χ^2 distributed with $(h_1 - h_0)$ df.

TABLE 1. Estimates of τ for the four species combinations in 1984 and 1985, based on a maximum of 26 replicate measurements of CPUE at 16 sites in 1984 and 50 replicate measurements of CPUE at 15 sites in 1985.

	τ	
Data set	1984	1985
CO	1.3503	1.1199
QB	0.7112	0.9053
RF	1.0722	1.4717
NC	0.6537	1.4274

The hierarchy of cases for model 1 and 2 is shown in Fig. 3, where cases are joined with a solid line when the lower case can be taken as a null hypothesis in relation to the upper case as an alternative hypothesis. Cases 1.4 and 2.4 are joined by a dotted line to indicate that they are identical if conditions (1a) and (3a) both apply. This hierarchy is notably more complex than that in Richards and Schnute (1986). In particular, there are two possible starting points at the bottom level, and the appropriate branch of the hierarchy must first be established. A modified top-down approach that is necessary to accommodate this hiearchy is as follows.

Step 1. Find parameter estimates and minimum values of A and A' for all cases. Examine the residuals and eliminate cases if the solution approximates a step function (i.e. when the solution is against the boundary of the constraint (2b) or (4b)).

Step 2. Case 1, 1.3, and 2.3 each contain one free parameter. Select the case of these three with the lowest minimum A or A'.

Step 3. If case 1 is selected in step 3, then go to step 4. If a case 3 is selected, then use B in (13) to test the case 3 against the corresponding case 5. If the case 3 cannot be rejected, then

use B to test the case 3 against the corresponding case 4. If the case 3 cannot be rejected by either test, then accept the case 3 and stop; otherwise, use B to test the corresponding case 4 against the case 5. If the case 4 is rejected, then accept the case 5; otherwise, accept the case 4.

Step 4. If case 1 is selected in step 2, then use B in (13) to test case 1 against the case 5 with the lowest minimum A or A'. If case 1 cannot be rejected, then use B to test case 1 against the case 2 or 4 with the lowest minimum A or A'. If case 1 cannot be rejected by either test, then accept case 1 and stop; otherwise, select the case 5 with the lowest minimum A or A' and the corresponding case 2 or 4 with the lowest minimum A or A'. Use B to test the case 2 or 4 against the case 5. If the case 2 or 4 is rejected, then accept the case 5; otherwise, accept the case 2 or 4.

Results

Data Standardization

Angling and SCUBA data sets include different sizes and species of fish. A standardization process was required before the data sets could be compared. Species such as spiny dogfish and rock sole (*Lepidopsetta bilineata*) were caught by anglers but were excluded from the analysis, as their densities could not be adequately assessed by divers. Conversely, species such as pile perch (*Rhacochilus vacca*) were observed by divers but were excluded, as they were not caught by anglers. The analysis included all rockfish species observed by divers or caught while angling, as well as lingcod (*Ophiodon elongatus*) and kelp greenling (*Hexagrammos decagrammus*). Rockfish species included copper rockfish (*Sebastes caurinus*), quillback rockfish, tiger rockfish (*S. nigrocinctus*), and yelloweye rockfish (*S. ruberrimus*). The minimum sizes (fork length) of fish that were caught by the angling gear were 20 cm for rockfish, 35 cm for lingcod, and 30 cm for kelp greenling. To compensate for angling selectivity, counts of fish of the minimum size or greater were used for density calculations.

In order to calculate densities from the transect counts, it was first necessary to estimate transect width. During 1985 transects, lingcod and kelp greenling were always sighted within 3.5 m of the transect line, and tiger rockfish were always within 2.5 m. For other rockfish, the distance varied with visibility. The proportion of rockfish sighted within 2.5 m of the transect line decreased from 100 to 96, 88, 69, and 48% as the visibility increased from less than 5 to 5, 6, 7, and 8 m or more. Hence, 1985 densities were based on the estimated number observed within 2.5 m of the transect line (a 5-m section) for rockfish and within 3.5 m of the line (a 7-m section) for kelp greenling and lingcod. Similar distance estimates were not made for 1984, and I applied the above corrections to the 1984 counts. Counts of most species were higher in 1984, perhaps due to differences in visual acuity of the divers, and I based the estimates on a 7-m transect section for all species. Densities used in the analysis had units of fish per 100 m^2.

Copper rockfish and quillback rockfish were the dominant species both observed by divers and caught by anglers (Fig. 4). Most rockfish did not appear to be affected by the presence of the divers. However, lingcod were occasionally disturbed by divers and kelp greenling were occasionally attracted to divers, so that densities may be underestimated or overestimated, respectively, for these species. The decrease in estimated densities between 1984 and 1985 is probably an artifact of minor differences in survey technique, survey dates, or interpretation of visibility estimates, rather than a true density decrease. Except for copper rockfish, differences in CPUE between years were not significant, as would be expected if the estimated changes in density were representative of changes in abundance. Copper rockfish CPUE was underestimated in 1984 due to a vessel effect (Richards et al. 1985a). Conversely, more yelloweye rockfish were caught than expected based on the density of other rockfish in 1984 and 1985. Yelloweye rockfish density increases with depth (Richards 1986a), and a wider depth range was covered by angling than by SCUBA.

The analysis was performed for four sets of density and CPUE data from each year, using different species combinations. These were (1) copper rockfish density and CPUE (CO), (2) quillback rockfish density and CPUE (QB), (3) combined rockfish density and CPUE (RF), and (4) rockfish, lingcod, and kelp greenling density and CPUE, but excluding copper rockfish catch (NC). Copper rockfish catch was excluded from data set NC because of poor CPUE estimates for copper rockfish and because the inclusion of copper rockfish obscured trends apparent for other species. Copper rockfish are found predominantly at depths above 20 m. The number caught by angling depended on the allocation of fishing effort over approximately 5–20 m within the 5 to 40-m depth range. This allocation was not consistent among sites or between years.

Data Analysis

In the analytical methods section above, it was assumed that the values of the power transformations (β, γ) and the variance weights (f_i, g_i) were known. These can be estimated from the data because of replication of CPUE and density measurements at a site. To choose parameter values required for the analysis, I first examined residual plots of the density data with β values of 0.1, 0.2, 0.3, 0.4, and 0.5 for all data sets. Residuals were approximately normally distributed with $\beta = 0.4$ for the 1984 data and $\beta = 0.3$ for the 1985 data. The standard deviation of transformed density estimates at a site was then chosen as the best estimate of the weighting factor f_i. If a site mean was zero, the lowest nonzero standard deviation for the data set was used as an estimate of f_i for that site. Equations (A.1) and (A.4)–(A.6) in Richards and Schnute (1986) were applied to the transformed densities to estimate σ. Here, σ is the correction for the site mean standard deviation from the sample standard deviation. As there were always 10 replicates at a site, $\sigma = \sqrt{0.1}$ for all data sets. CPUE residuals were approximately normally distributed with $\gamma = 1$, and u_i, τ (Table 1), and g_i were estimated from equations (A.9) and (A.11)–(A.15) of Richards and Schnute (1986).

Because the model is symmetrical, it can be applied to examine the shape of the relationship between different abundance indices in one year, or between one abundance index in different years. For example, it can be applied to test whether changes in an abundance index occur independently among sites in different years. To compare the 1984 and 1985 CPUE indices, for example, I let U represent the CPUE in 1985 and D represent the CPUE in 1984. To compare the 1984 and 1985 density indices, I let U represent the density in 1985 and D represent the density in 1984. For convention, I chose the prediction of 1985 values from 1984 data as model 1 and the prediction of 1984 values from 1985 data as model 2.

TABLE 2. Parameter estimates and minimum A and A' values for five cases of models 1 and 2 for the relationship between selected CPUE and density indices. As σ and τ are identical for the density comparisons, only σ is given.

Case	Model 1						Model 2					
	p	q	r	σ	τ	min A	p[a]	q	r	σ	τ	min A'
	CPUE on density						*Density on CPUE*					
	1984 quillback rockfish (QB)											
1	—	0.042	—	0.305	0.687	−6.189[b]	—	0.042	—	0.305	0.687	−6.189[b]
2	—	0.044	1.30×10^{-3}	0.305	0.686	−6.197	—	0.042	—	0.305	0.687	−6.189
3	0.46	—	—	0.438	0.986	1.777	12.2	—	—	0.416	0.935	0.607
4	0.08	0.038	—	0.303	0.682	−6.328	0.08	0.038	—	0.303	0.682	−6.328
5	0.08	0.038	—	0.303	0.682	−6.328	0.09	0.036	-8.32×10^{-4}	0.303	0.682	−6.332
	1985 copper rockfish (CO)											
1	—	0.417	—	0.330	1.17	0.571[b]	—	0.417	—	0.330	1.17	0.571[b]
2	—	0.417	—	0.330	1.17	0.571	—	-1.33×10^{-5}	0.130	0.273	0.97	−4.026[c]
3	3.35	—	—	1.127	3.99	30.052	3.87	—	—	0.336	1.19	0.981
4	−44.0	5.69	—	0.274	0.97	−3.885[c]	−2.49	1.21	—	0.329	1.17	0.525
5	−4.40	5.69	—	0.274	0.97	−3.885[c]	—	-1.33×10^{-5}	0.130	0.273	0.97	−4.026[c]
	1985 combined rockfish (RF)											
1	—	0.104	—	0.541	2.52	15.69	—	0.104	—	0.541	2.52	15.69
2	—	0.104	—	0.541	2.52	15.69	—	3.86×10^{-5}	−0.021	0.484	2.25	13.06[c]
3	4.98	—	—	0.980	4.56	29.97	31.3	—	—	0.521	2.43	14.83[b]
4	0.85	0.086	—	0.533	2.48	15.35	31.3	—	—	0.521	2.43	14.83
5	0.85	0.086	—	0.533	2.48	15.35	—	3.86×10^{-5}	−0.021	0.484	2.25	13.06[c]
	Density: 1985 on 1984						*Density: 1984 on 1985*					
	Copper rockfish (CO)											
1	—	0.282	—	0.246		−19.87	—	0.282	—	0.246		−19.87
2	—	0.334	4.14×10^{-3}	0.244		−20.07	—	0.282	—	0.246		−19.87
3	3.92	—	—	0.350		−12.07	17.3	—	—	0.367		−11.06
4	−1.97	0.432	—	0.225		−21.87	−1.95	0.429	—	0.225		−21.87
5	−56.5	7.232	0.269	0.190		−25.58[b]	−1.95	0.429	—	0.225		−21.87
	CPUE: 1985 on 1984						*CPUE: 1984 on 1985*					
	Copper rockfish (CO)											
1	—	2.63	—	2.25	1.86	29.19[b]	—	2.63	—	2.25	1.86	29.19[b]
2	—	2.63	—	2.25	1.86	29.19	—	0.34	−0.425	2.11	1.75	27.72
3	3.55	—	—	4.55	3.77	46.10	1.08	—	—	2.30	1.91	29.73
4	−2.32	4.07	—	2.22	1.84	28.91	−2.18	3.98	—	2.22	1.84	28.93
5	−2.32	4.07	—	2.22	1.84	28.91	0.94	3.50×10^{-6}	−0.367	2.07	1.72	27.26
	Combined rockfish (RF)											
1	—	1.75	—	2.51	3.45	37.91[b]	—	1.75	—	2.51	3.45	37.91[b]
2	—	4.53	0.329	2.39	3.29	36.76	—	1.75	—	2.51	3.45	37.91
3	5.34	—	—	2.97	4.07	41.90	1.81	—	—	3.25	4.46	44.06
4	1.55	1.33	—	2.49	3.42	37.68	2.36	1.11	—	2.48	3.41	37.61
5	-1.94×10^{6}	2.20×10^{6}	1.98×10^{5}	2.29	3.14	35.66	2.36	1.11	—	2.48	3.41	37.61

[a] p' when $q = 0$.
[b] Case selected by the hypothesis testing sequence at the 0.05 significance level.
[c] Step function, case rejected by residual tests.

Parameter estimates for models 1 and 2 were obtained by the simplex search method (Mittertreiner and Schnute 1985) for the five cases of the eight CPUE–density comparisons, four 1984–85 CPUE comparisons, and four 1984–85 density comparisons. The main features of the solutions are illustrated by CPUE–density data sets 1984 QB, 1985 CO, and 1985 RF, CPUE data sets CO and RF, and density data set CO (Table 2). Only the best solution is reported for each case, although multiple solutions were found for some cases. Note that case 1 is identical for both models. Either case 1.2 or 2.2 is against the constraint (2a) or (4a), respectively, and is identical to case 1. Similarly, case 1.5 or 2.5 is against the constraint (2a) or

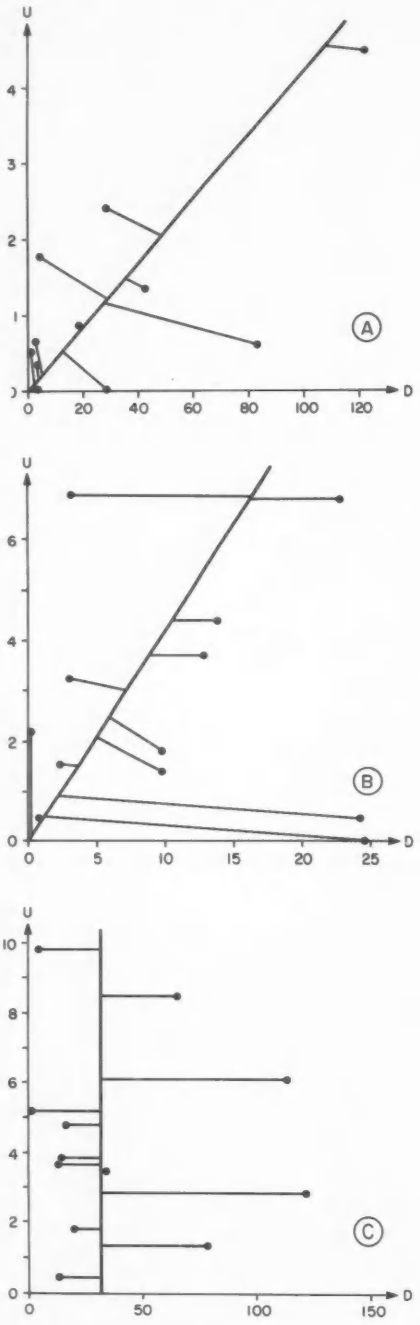

FIG. 5. Solutions for the relationship between CPUE (U, fish·line^{-1}·h^{-1}) and the density index (D, fish·(100 m^2)$^{-1}$). The thin lines connect data points to the corresponding estimates of density and CPUE on the curve. (A) 1984 QB (case 1); (B) 1985 CO (case 1); (C) 1985 RF (case 2.3).

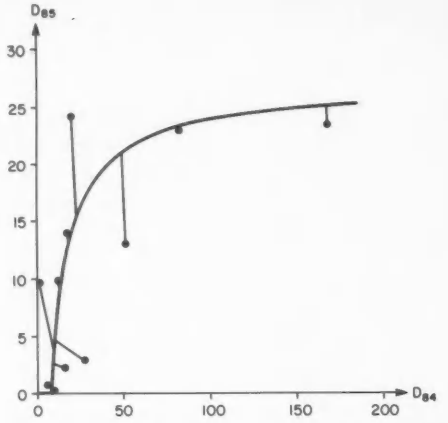

FIG. 6. Case 1.5 solution for the relationship between the 1984 CO density index (D_{84}, fish·(100 m^2)$^{-1}$) and the 1985 CO density index (D_{85}, fish·(100 m^2)$^{-1}$). The thin lines connect data points to the corresponding density estimates on the curve.

(4a), respectively, and is identical to the better of the corresponding case 2 or 4.

For CPUE–density comparisons 1985 CO and 1985 RF, the solutions to one or more of the cases lie against the constraint (2b) or (4b), and approximate a step function. These cases were rejected by step 1 of the hypothesis testing sequence, as the residuals were either near zero or large. Examples of similar solutions are shown in fig. 4 of Richards and Schnute (1986). In addition, for 1985 RF, the solution to case 2.3 and 2.4 is identical. The slope of the line in case 4 is constrained to be positive and the solution lies on this constraint.

Minimum A or A' values are an index of goodness of fit for cases within a data set and can also provide a rough comparison among data sets. It is obvious from Table 2 that case 1.3 can be rejected for all data sets on the basis of high A values. For CPUE–density comparisons, case 1.3 corresponds to a constant CPUE. In the absence of model 2, its rejection implies that a relationship of some form is present between the CPUE and density data. This is not true for case 2.3, however, and for 1985 RF (Fig. 5C), case 2.3 is a better solution than case 1. With higher variance in the density data, a constant density is a more likely solution than is a constant CPUE.

For CPUE–density comparisons 1984 QB and 1985 CO, the accepted solution at the 0.05 significance level is one of strict proportionality (Fig. 5A, 5B). There is a high degree of confidence in the 1984 QB solution because of the low values of σ, τ, and A. In contrast, the shape of the relationship for 1985 CO is poorly determined. The A' values for cases 1 and 2.3 are relatively close and there is no acceptable case 5 solution. With high variation in the density data, a hypothesis of strict proportionality cannot be rejected. The solutions to CPUE comparisons CO and RF are also poorly determined. For CO, there is more variation in the 1984 CPUE data compared with more variation in the 1985 CPUE data for RF. The lowest solutions are for cases 2.5 and 1.5 for the two data sets, respectively.

For the density comparisons, case 1.5 is the accepted solution for all data sets. The best fit is for CO (Fig. 6). Over the median range of densities, the relationship between 1984 and 1985 densities is approximately linear. However, density esti-

mates in 1985 are disproportionally lower at low, and the two-three high density sites. It is obvious that the density index has changed between years, either as a result of differences in methodology, site-specific movements of fish, or both. Density estimates tended to have a higher variance in 1985 than in 1984, and there was a higher frequency of zero density recorded on the 1985 transects.

It is important to recognize that failure to reject the null hypothesis does not imply that the null hypothesis is true. When the true relationship between CPUE and density is asymptotic or has a nonzero intercept, failure to reject case 1 can be especially treacherous to fishery managers. The true decline of a stock could go undetected. One way to examine the confidence in the accepted solution is to compare the outcomes over a range of significance levels. The probability of making a type II error tends to decrease as the significance level of the test is relaxed.

To examine further the confidence in the accepted solutions, I varied the significance level of the B statistic from 0.05 to 0.80 and compared the accepted solutions (Table 3). Except where both case 5 solutions are rejected by residual tests, case 5 is the accepted solution at the 0.50 significance level for all but two of the data sets. The CPUE comparisons CO and RF that appeared from Table 2 to be poorly determined have asymptotic (case 2) solutions at the 0.10 significance level. Conversely, there is a relatively high degree of confidence in the case 1 solutions for the data sets that include QB CPUE. Of course, once a case 5 solution is accepted, for example for the density comparisons, then relaxing the significance level will not affect the outcome. Whether case 1.5 or 2.5 is ultimately accepted depends on λ and the weighting factors f_i and g_i.

TABLE 3. Accepted solutions to all data sets as determined by the B statistic at significance levels of 0.05–0.80.

Data set	Significance level				
	0.05	0.10	0.20	0.50	0.80
CPUE–density comparisons					
1984 CO	2.3[a]	2.3	2.3	2.3	2.3
1984 QB	1	1	1	1	4[b]
1984 RF	2.3	2.5	2.5	2.5	2.5
1984 NC	1.5	1.5	1.5	1.5	1.5
1985 CO	1[a]	1	1	1	2.4
1985 QB	1	1	2.5	2.5	2.5
1985 RF	2.3[a]	2.3	2.3	2.3	2.3
1985 NC	1	2.5	2.5	2.5	2.5
CPUE comparisons					
CO	1	2.2	2.2	2.5	2.5
QB	1	1	1.4	1.4	1.5
RF	1	1.2	1.5	1.5	1.5
NC	1.5	1.5	1.5	1.5	1.5
Density comparisons (all data sets)					
	1.5	1.5	1.5	1.5	1.5

[a]Case 5 solutions rejected by residual tests.
[b]Case 1.4 and 2.4 solutions are identical.

Discussion

In this paper, I have generalized the model of Richards and Schnute (1986) and extended its application. The model can be used to relate any pair of variables if an estimate of the ratio of variances of the variables is available. This is possible because the model has symmetric counterparts in which the two variables are reversed. Here, I used the model to relate two sets of density indices, two sets of CPUE values, and two sets of CPUE–density combinations. I used density estimates of nearshore reef fishes from the Strait of Georgia, British Columbia, obtained by SCUBA, and CPUE estimates obtained by angling at the same sites.

For quillback rockfish, Richards and Schnute (1986) found a relationship between CPUE and density of strict proportionality. Quillback rockfish densities were included in their only single-species data set. The shape of the relationship was less well determined for their multispecies data sets. They suggested that some of the ambiguity in the shape of the relationship could be reduced with an increased number of replicates. This will only be true, of course, if an increased number of replicates reduces the sample variance. The density data and the 1985 CPUE data used here had more replicates than the comparable data sets in Richards and Schnute (1986). However, the shape of the 1985 relationships was still relatively poorly determined due to the higher variance of the 1985 density data relative to the 1984 density data. For copper rockfish and for species combinations that included copper rockfish, there was either no relationship, or the shape of the relationship was poorly determined. Conversely, for the 1984 multispecies data set NC, the relationship was well determined and was asymptotic with both low and high density departures from proportionality.

By restricting their analysis to model 1, Richards and Schnute (1986) implicitly assumed that visual density estimates were a more accurate representation of true abundance than were estimates of CPUE. Neither index is preferred in the analysis presented here, and it is possible to examine this assumption. It could be argued that CPUE is, in fact, a better abundance index, based on the observed relationship between CPUE and density. This is because density estimates tended to have a higher variance and a constant density was an accepted solution at the 0.05 significance level for three of the data sets. Conversely, for the between-year relationships, it is apparent that density is a more consistent abundance index, at least for data sets that include copper rockfish. Of course, this is all somewhat hypothetical, as the true abundances are not known.

For mobile fish species, there may be no reason to expect consistently higher abundances at some sites, or that between-year differences in abundance will be in strict proportion among sites. However, for data sets that did not include copper rockfish catch, there was a clear relationship for both between-year CPUE estimates and between-year density estimates at a site. Such a result could occur if fishing conditions or conditions for making visual density estimates were similar at a site in both years, but differed among sites. The shape of the relationships could also result from minor differences in methodology between years or from fish behavior.

For rockfish stock assessment purposes, it is encouraging that the relationship of strict proportionality found for quillback rockfish over depths of 41–70 m (Richards and Schnute 1986) also held for experiments in two years over a

shallower depth range (5–40 m) using a different abundance index. Quillback rockfish is the dominant species in the commercial inshore rockfish fishery (Cass et al. 1986). At present, there is little information other than CPUE from the commercial fishery on which to base stock assessments (Richards 1986b).

The management value of CPUE as an abundance index depends on the shape of the relationship between CPUE and abundance as well as on the associated level of confidence. If visual estimates are indeed accurate, if the accepted solution is case 2.2 or 2.5, and if the estimated range of abundances is close to the asymptote, then CPUE will not be a useful abundance index. It is not reasonable to expect CPUE to increase indefinitely at a constant density. If this appears to occur, then a model that allows for a sigmoidal relationship, such as a growth model (Schnute 1981), may be more appropriate than equation (1). The analytical ideas presented here could easily be transferred to another such class of models.

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Optimal Policies for Rehabilitation of Overexploited Fish Stocks Using a Deterministic Model

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Hightower, J. E., and G. D. Grossman. 1987. Optimal policies for rehabilitation of overexploited fish stocks using a deterministic model. Can. J. Fish. Aquat. Sci. 44: 803–810.

Traditional fisheries models are of limited use during the transitional period when effort is reduced to permit recovery of an overexploited stock. We used a generalized age-structured model to represent stock dynamics during this transitional period and obtained optimal harvesting policies for three overexploited fish stocks using a first-order gradient procedure. The length of the rehabilitation period was affected by (1) the demographic characteristics of the stock, (2) the historical level of exploitation, and (3) the form of the objective function. When a target stock biomass or spawning stock was specified, rehabilitation was relatively rapid due to the sharp initial reduction in fishing effort. Fishing began earlier in the planning horizon when a target harvest was specified; however, stocks recovered more slowly and fishing effort and harvest did not stabilize at the desired levels as rapidly. Policies for maximizing harvest did not usually result in closure of the fishery; however, fishing effort usually exceeded the desired level and sometimes fluctuated considerably from year to year. Our results should aid in the development of rehabilitation policies tailored to specific fisheries or specific management goals.

L'application de modèles traditionnels de pêche est limitée pendant la période de transition quand l'effort est réduit afin de permettre le rétablissement d'un stock surexploité. Les auteurs utilisent un modèle généralisé basé sur l'âge pour représenter la dynamique d'un stock pendant cette période de transition; ils ont ainsi obtenu des plans d'exploitation optimale pour trois stocks surexploités à l'aide de fréquences du premier ordre. La longueur de la période de rétablissement a varié selon les facteurs suivants : 1) les caractéristiques démographiques du stock, 2) le niveau historique d'exploitation et 3) la forme de la fonction objective. La détermination de niveaux cibles pour la biomasse du stock ou le stock reproducteur a permis un rétablissement assez rapide à cause de la forte baisse initiale de l'effort de pêche. Dans le cadre de la planification, la pêche a commencé plus tôt quand on a précisé le niveau cible d'exploitation; toutefois, la récupération des stocks a été plus lente et l'effort de pêche et les captures ne se sont pas stabilisés aussi rapidement au niveau désiré. En général, les plans de maximalisation des captures n'ont pas entraîné une fermeture de la pêche; toutefois, l'effort de pêche a généralement dépassé le niveau désiré et a parfois varié fortement d'une année à l'autre. Les résultats obtenus devraient servir à l'élaboration de plans de rétablissement adaptés à des pêches précises ou à des buts de gestion déterminés.

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Alverson and Paulik (1973) noted that traditional management models, such as the Beverton–Holt yield-per-recruit model (Ricker 1975), were of limited value for management of developing fisheries because these fisheries typically have not reached steady-state conditions. The same limitation also applies to management of overfished stocks. The latter case is of considerable interest because many stocks are fully exploited or have been overharvested (Cushing 1981). For example, Atlantic menhaden (*Brevoortia tyrannus*) and Pacific ocean perch (*Sebastes alutus*) stocks have been overfished for several years and reductions in fishing effort have been recommended (Schaaf 1979; Archibald et al. 1983; Pacific Fishery Management Council 1984). To facilitate rehabilitation of overexploited stocks, management models are needed to provide quantitative recommendations for the transitional period to lower fishing effort.

Age-structured population models have been used to develop rehabilitation policies for several overexploited fish stocks. For example, Huang and Walters (1983) used an age-structured model to compare short-term approaches for rehabilitation of a large yellow croaker (*Pseudosciaena crocea*) stock. In their study, alternative strategies were selected arbitrarily and compared through simulation studies. Stock rehabilitation required about 15 yr under a constant effort policy, but occurred much more rapidly if the fishery was closed for 3 yr. Ruppert et al. (1985) used an age-structured model to develop optimal rehabilitation policies for the Atlantic menhaden stock. They found that the stock was restored to the optimal steady-state level in about 9 yr using either a constant effort policy or one of four "egg escapement" policies. The steady-state performance of the five policies was somewhat similar, although there were notable differences in harvest during the rehabilitation period. Archibald et al. (1983) compared several constant effort policies for rehabilitation of a Pacific ocean perch stock. Results for a 30-yr planning horizon were used to evaluate the "short-term"

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performance of these policies; however, simulation runs of 100 yr or more were required to achieve steady-state levels.

These results demonstrate that the life history characteristics of the stock and the form of the harvesting policy can have important effects on the management strategies employed during the rehabilitation period. Our objective in this study was to examine the effects of planning horizon length, stock demographic characteristics, and objective function type on the form of optimal rehabilitation policies for three overexploited fish stocks. Our results can be used to develop rehabilitation policies tailored to specific fisheries; for example, a policy with gradually decreasing effort levels might be preferable to one that achieves stock rehabilitation quickly.

Methods

Age-structured models have been used to simulate a variety of fisheries, including South African anchovy (*Engraulis capensis*) (Getz 1980, 1985), Atlantic menhaden (Schaaf 1979; Ruppert et al. 1985), Pacific ocean perch (Archibald et al. 1983), large yellow croaker (Huang and Walters 1983), and Arcto-Norwegian cod (*Gadus morhua*) (Walters 1969). The model used in this study was a slightly generalized version of a deterministic model presented by Getz (1980), who assumed that each simulated year consisted of a fishing season followed by a spawning season. We assumed that each year can be divided into a fishing season of length t' and a closed season of length $(1 - t')$. Spawning was assumed to occur either at the beginning of the year or after the fishing season. Following Getz (1980), we assumed that

$$(1)\quad x_{i+1}(k+1) = x_i(k)\exp(-M_i - q_i f(k)t') \qquad (k = 0,\ldots,N-1;\ i = 1,\ldots,\lambda-2)$$

$$(2)\quad x_\lambda(k+1) = x_{\lambda-1}(k)\exp(-M_{\lambda-1} - q_{\lambda-1} f(k)t') + x_\lambda(k)\exp(-M_\lambda - q_\lambda f(k)t')$$

where $x_i(k)$ = number of age i fish in year k, λ = maximum age class used in the model, t' = length of the fishing season, q_i = catchability coefficient for age i fish, M_i = annual instantaneous rate of natural mortality for age i fish, and $f(k)$ = level of fishing effort in year k. The stock–recruitment model was either of the form proposed by Ricker (1975):

$$(3)\quad x_1(k+1) = \alpha S(k)\exp(-\beta S(k))$$

or Beverton and Holt (1957):

$$(4)\quad x_1(k+1) = 1/(\alpha + \beta/S(k))$$

where α and β were parameters for either the Ricker or Beverton–Holt curve and $S(k)$, the spawning stock in year k, was defined as

$$(5)\quad S(k) = \sum_{i=1}^{\lambda} c_i x_i(k)$$

when spawning occurred at the beginning of the year or

$$(6)\quad S(k) = \sum_{i=1}^{\lambda} c_i x_i(k)\exp(-t'(M_i + q_i f(k)))$$

when spawning occurred after the fishing season, where c_i = fecundity index for age i fish. Harvest in year k was defined as

$$(7)\quad h(k) = \sum_{i=1}^{\lambda} x_i(k) w_i q_i f(k)(1 - \exp(-t'(M_i + q_i f(k)))) \div (M_i + q_i f(k))$$

where w_i = weight of age i fish. It should be noted that average weight-at-age may change if optimal and current levels of fishing mortality differ markedly (Ricker 1975). For example, if the mortality rate is reduced under the optimal policy, mean weight-at-age should increase because more fish survive to the larger sizes reached later in the year (Ricker 1975). Density-dependent changes in weight-at-age also might be observed if stock size changes substantially over the planning horizon. These factors may bias long-term estimates of the optimal levels of fishing effort; however, the practical consequences of this result are small if revised estimates of weight-at-age are used to update the policy periodically.

We examined harvesting policies obtained by minimizing

$$(8)\quad \sum_{k=0}^{N-1} (b(k+t') - b^*)^2$$

where b^* represented the target stock biomass at the end of the fishing season and $b(k + t')$ was defined as

$$(9)\quad b(k+t') = \sum_{i=1}^{\lambda} x_i(k) w_i \exp(-t'(M_i + q_i f(k))).$$

To determine the target stock biomass, we assumed that an appropriate level of fishing effort for the rehabilitated stock would be $f_{0.1}$, the level at which the slope of the yield–effort curve was 1/10 the slope of the curve at the origin. This marginal yield criterion frequently has been used in yield per recruit analyses to select an appropriate fishing mortality rate (Sissenwine 1981). This recommended rate of fishing mortality may be considerably lower than the rate needed to obtain maximum sustained yield (MSY), yet the loss in yield may be minor (Sissenwine 1981). In addition to greater economic efficiency (Gulland and Boerma 1973), an additional advantage of this criterion is the maintenance of a larger spawning stock (Sissenwine 1978). Similar advantages should apply when used with yield–effort curves; therefore, we defined b^* as the equilibrium stock biomass obtained at $f_{0.1}$.

The second set of harvesting policies was obtained by minimizing

$$(10)\quad \sum_{k=0}^{N-1} (s(k+t') - s^*)^2$$

where s^* represented the target spawning stock at the end of the fishing season. We defined s^* as the equilibrium spawning stock obtained at $f_{0.1}$ and obtained $s(k + t')$ from (9) by replacing w_i with c_i. Note that $s(k + t')$ is equivalent to $S(k)$ in (6) if spawning occurs after the fishing season.

The third set of harvest policies was obtained by minimizing

$$(11)\quad \sum_{k=0}^{N-1} (h(k) - h^*)^2 + \delta \sum_{i=1}^{\lambda} (x_i(N) - x_i^*)^2$$

where h^* was the equilibrium harvest obtained at $f_{0.1}$, δ was a penalty weight, and $\boldsymbol{x}^*$ (the target number-at-age vector at time N) was defined as the equilibrium number-at-age vector at $f_{0.1}$. Walters (1975) obtained harvesting strategies for Pacific salmon by minimizing

$$(12)\quad \sum_{k=0}^{N-1} (h(k) - h^*)^2$$

and suggested that those strategies conformed more closely to actual management practices than did strategies for harvest maximization. Stock rehabilitation policies that we obtained using (12) were unacceptable, however, for two reasons. First,

TABLE 1. Equilibrium number-at-age vectors obtained at the maximum sustainable level of fishing effort (f_{max}) and twice the level designated as optimal ($f_{0.1}$).

Stock	Historical level of effort	Age class	Number per age class							
Anchovy	f_{max}	1–5+	0.6	0.2	0.0	0.0	0.0			
	$2 \times f_{0.1}$		61.5	20.7	6.0	1.6	0.3			
Atlantic menhaden	f_{max}	1–8+	0.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	$2 \times f_{0.1}$		2.8	1.4	0.3	0.1	0.0	0.0	0.0	0.0
Pacific ocean perch	f_{max}	1–8	28	28	28	28	28	28	27	25
		9–16	24	22	20	18	15	13	11	9
		17–24	8	7	6	5	4	4	3	3
		25–29+	3	2	2	2	13			
	$2 \times f_{0.1}$	1–8	118	118	118	118	118	118	111	105
		9–16	99	91	83	75	65	56	48	41
		17–24	35	30	27	24	21	19	17	15
		25–29+	13	11	10	9	70			

any given equilibrium harvest (other than MSY) could be obtained at two different levels of fishing effort (i.e. one above and one below MSY). Stock size was lower at the higher effort level and this was the equilibrium attained by the rebuilding stock. A second problem arose with the use of the scalar quantity h^* as the target harvest from a multi-aged stock. This harvest can be obtained from more than one number-at-age vector, and harvests approximating h^* were sometimes obtained by increasing effort to offset decreases in stock size. We assumed that an appropriate rehabilitation strategy was one leading to a period of stable effort and harvests and incorporated an end point penalty (Getz 1985) into (11) to accomplish this result. Note that policies obtained from (11) or (12) could be inappropriate in some instances because deviations from h^* are treated equally, regardless of direction. Even if stability in harvest is a primary objective, most fishermen and managers would consider harvests larger than h^* to be much less of a problem than harvests smaller than h^*. An alternative approach would be to give less weight to those deviations where harvest exceeds h^*. We did not evaluate that approach but would not expect our results to change substantially because harvests generally would be less than h^* during the rehabilitation period.

The fourth set of harvesting policies was obtained by maximizing

$$(13)\quad \sum_{k=0}^{N-1} h(k) - \delta \sum_{i=1}^{\lambda} (x_i(N) - x_i^*)^2$$

where the end point penalty was used to prevent the stock from being depleted at the end of the planning horizon. A fifth set of harvesting policies was obtained by maximizing $\Sigma \log (h(k) + 1)$, using the same end point penalty as in (13). Ruppert et al. (1985) suggested that a policy for maximizing log (harvest) might be appropriate whenever there is substantial aversion to risk. In our deterministic analysis, however, policies for maximizing harvest and log (harvest) usually were similar; hence, results for the log (harvest) criterion will not be presented herein.

Fishing effort in our analysis was permitted to vary from 0 to f_{max}, where f_{max} was chosen to be the greatest level of fishing effort at which a nonzero equilibrium was attained by the simulated stock. Note that f_{max} also could represent fleet capacity (Ludwig 1981).

We obtained optimal rehabilitation policies by using a first-order gradient procedure (Dyer and McReynolds 1970; Dreyfus and Law 1977; Getz 1985). (A listing or copy of a microcomputer-based version is available from the senior author.) We assumed that the initial state of the system $\boldsymbol{x}(0)$ was known; for example, through application of a stock reconstruction technique such as cohort analysis (Pope 1972). The optimal rehabilitation policy was defined as the N levels of fishing effort $f(0), \ldots, f(N-1)$ that minimized (8), (10), or (11), or that maximized (13), subject to state transformation equations (1)–(6). We obtained the optimal decision sequence by iteratively improving the value of an arbitrary initial sequence $f^0(0), \ldots, f^0(N-1)$ until increases in value were negligible (Dyer and McReynolds 1970; Dreyfus and Law 1977). Changes in the decision sequence were obtained by first expressing the value in year k, $T(k)$, as a recursive formula. For example, the value of a policy for achieving a target harvest was obtained from

$$(14)\quad T(k) = -(h(k) - h^*)^2 + T(k+1) \qquad (k = 0, \ldots, N-1)$$

where $T(N) = -\delta \sum_{i=1}^{\lambda} (x_i(N) - x_i^*)^2$. The gradient vector $\partial T(k)/\partial f(k)$ was computed to determine the direction for changes in the decision sequence. At the jth iteration, the updated decision sequence was obtained as

$$(15)\quad f^j(k) = f^{j-1}(k) + c\partial T(k)/\partial f(k)$$

where c was chosen to maximize the increase in value (Dreyfus and Law 1977).

We determined stock rehabilitation policies for a South African anchovy stock, the Atlantic menhaden stock, and a Pacific ocean perch stock (see Hightower and Grossman (1985) and references therein). We obtained rehabilitation strategies for two hypothetical cases by assuming that the historical level of fishing effort was either f_{max} or $2 \times f_{0.1}$ (Table 1). The former case represented a fishery for a heavily overexploited stock, whereas the latter represented a fishery in which the stock was moderately overexploited. In all cases, references to management policies refer to policies for rehabilitation of these hypothetical stocks.

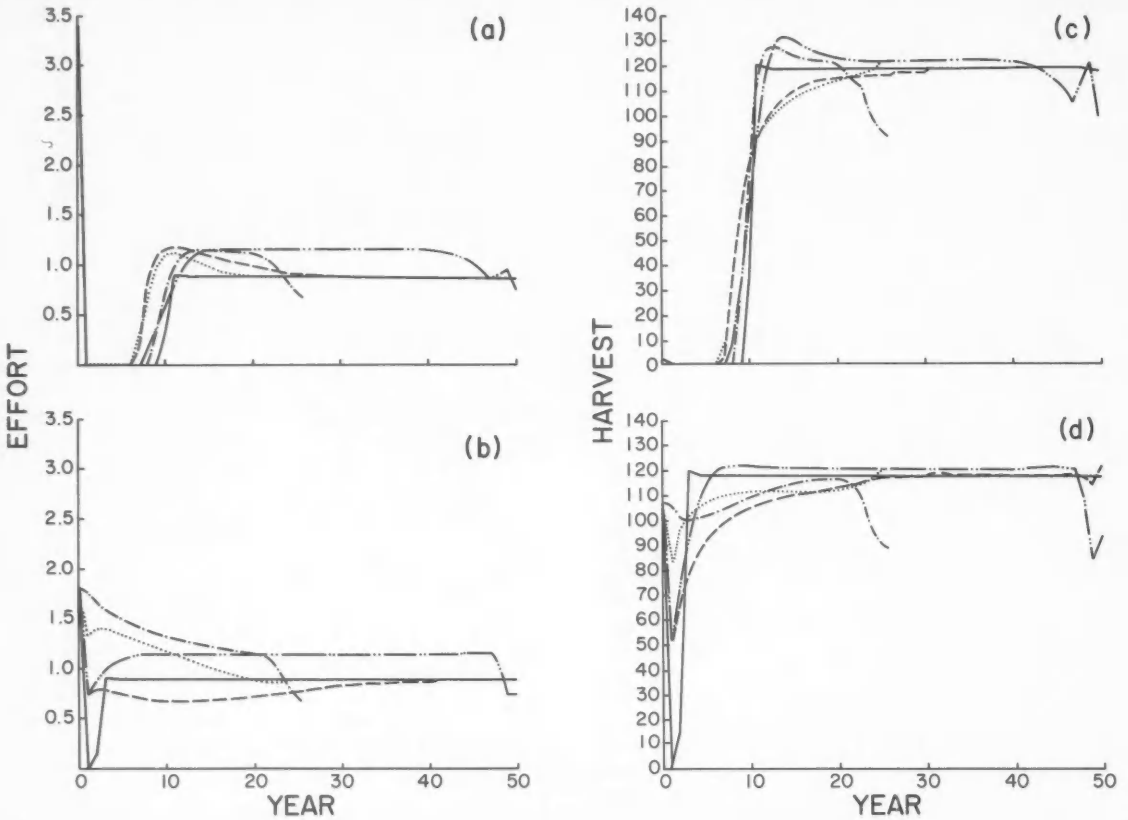

FIG. 1. Optimal levels of fishing effort and harvests for a (a, c) heavily depleted and (b, d) moderately depleted anchovy stock, obtained by maximizing harvest (−·−, 25 yr; −···−, 50 yr), specifying a target spawning stock s^* (—, 50 yr) or specifying a target harvest h^* (···, 25 yr; −−, 50 yr). The 25- and 50-yr policies for attaining s^* differed only in the length of the final constant-effort period, so only the latter policy is shown.

Results and Discussion

Two related problems in developing a policy for stock rehabilitation are (1) determining the length of the rehabilitation period and (2) selecting an appropriate level of annual effort during the rehabilitation period. We found that a planning horizon of two to three times the life span of the fish was sufficient for rehabilitation to occur. Note that these stocks had been fished at the maximum sustainable level, so a shorter rehabilitation period could be used in most instances.

Policies for achieving a target stock biomass and target spawning stock were almost identical for the three stocks examined in this study, so results for a target stock biomass were omitted from Fig. 1–3. Both policies resulted in rapid recovery of the overexploited anchovy and Atlantic menhaden stocks (Fig. 1 and 2). Even when heavily depleted, stocks recovered fully in 5–10 yr and effort was constant during the post-rehabilitation period. A 25-yr planning horizon was sufficiently long for rehabilitation to occur, and increasing the planning horizon to 50 yr merely increased the length of the final constant-effort period. Note that rehabilitation periods were longer for the shorter-lived anchovy stock ($\lambda = 5$) than for the Atlantic menhaden stock ($\lambda = 8$). The difference in the number of age classes is misleading, however, because few Atlantic menhaden lived beyond age 5 unless the level of fishing effort was low. A more important difference between the two stocks is that a Beverton–Holt curve was used to represent the anchovy stock–recruitment relationship, whereas a Ricker curve was used for Atlantic menhaden. This difference enabled the Atlantic menhaden stock to rebuild more quickly because maximum recruitment occurred at an intermediate stock size. For example, in the heavily exploited case, the Atlantic menhaden spawning stock increased to the level producing maximum recruitment in about 4 yr if the fishery was closed. In that same period, the anchovy spawning stock also increased substantially but recruitment produced by that spawning stock was only about one-fifth the maximum level. The Pacific ocean perch stock recovered much more slowly than did the anchovy and Atlantic menhaden stocks; hence, a planning horizon of 100 yr was used. About 60 yr without fishing were needed to rebuild the heavily depleted stock, whereas a 25-yr closure was needed when the stock was only moderately overexploited (Fig. 3). The optimal policy for all three stocks was to close the fishery until stock biomass or spawning stock exceeded the target level. Once the fishery reopened, harvests stabilized at the desired level (h^*) within a few years (Fig. 1–3).

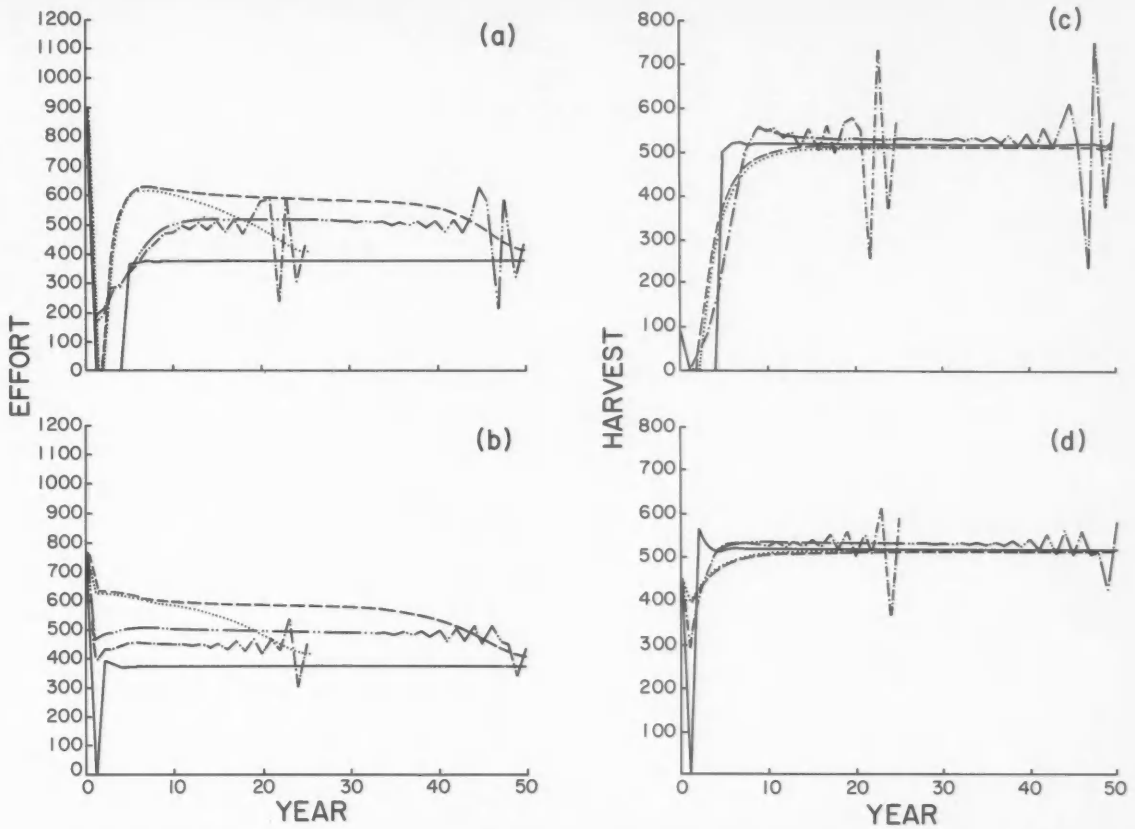

FIG. 2. Optimal levels of fishing effort and harvests for a (a, c) heavily depleted and (b, d) moderately depleted Atlantic menhaden stock, obtained by maximizing harvest (−·−, 25 yr; −···−, 50 yr), specifying a target spawning stock s^* (—, 50 yr) or specifying a target harvest h^* (···, 25 yr; −−, 50 yr). The 25- and 50-yr policies for attaining s^* differed only in the length of the final constant-effort period, so only the latter policy is shown.

Policies obtained by specifying a target harvest resulted in a more gradual recovery for all three stocks. Fishing began earlier in the planning horizon; however, target levels of fishing effort and harvest were not reached as rapidly (Fig. 1–3). For example, in comparison with results obtained through the use of a target biomass criterion, fishing for the moderately depleted Atlantic menhaden stock began 1 yr earlier, but harvests were lower in years 2–25. One disadvantage of target harvest policies was apparent in cases where the fishery was closed at the start of the planning horizon. Once the fishery was reopened, effort levels increased to above $f_{0.1}$ in order to obtain harvests approximating h^* (Fig. 1–3). Given the difficulty of reducing fishing effort, a more practical approach might be to permit effort to increase only to $f_{0.1}$ and then allow harvest levels to increase gradually to h^*. Policies for attaining a target harvest were affected by a change in the length of the planning horizon. By increasing the time available for convergence to the target vector $\boldsymbol{x}^*$, a more gradual reduction in fishing effort was sometimes possible (Fig. 2).

Policies for maximizing harvest appeared to be the most practical alternative in several of the cases considered herein. For example, the 25-yr policy for the moderately depleted anchovy stock required a gradual reduction in fishing effort to a level of about $f_{0.1}$. For the heavily depleted Atlantic menhaden stock and for both heavily and moderately depleted Pacific ocean perch stocks, harvest maximizing policies were the only management options that did not require closure of the fishery. In addition, harvests at the start of the rehabilitation period were only moderately below the historical level, which is shown in year 0 (Fig. 2d, 3c, 3d). The harvest maximizing policy for the heavily depleted Pacific ocean perch stock might be particularly useful if the species was taken incidentally in other fisheries, because some landings would be permitted throughout the planning horizon. The primary disadvantage of harvest maximizing policies was that, in the anchovy and Atlantic menhaden fisheries, effort frequently increased to the level producing maximum sustained yield (f_{msy}). This occurred in all cases when a 50-yr horizon was used, because convergence to $\boldsymbol{x}^*$ was achieved relatively quickly at the end of the planning horizon. The disadvantage of fishing at f_{msy} is illustrated in Fig. 1 and 2 in that only a slightly higher yield was obtained at a substantially higher effort level. An additional problem with harvest maximizing policies for Atlantic menhaden was that effort sometimes fluctuated substantially from year to year. This phenomenon only occurred near the end of the planning horizon; thus, its practical consequence

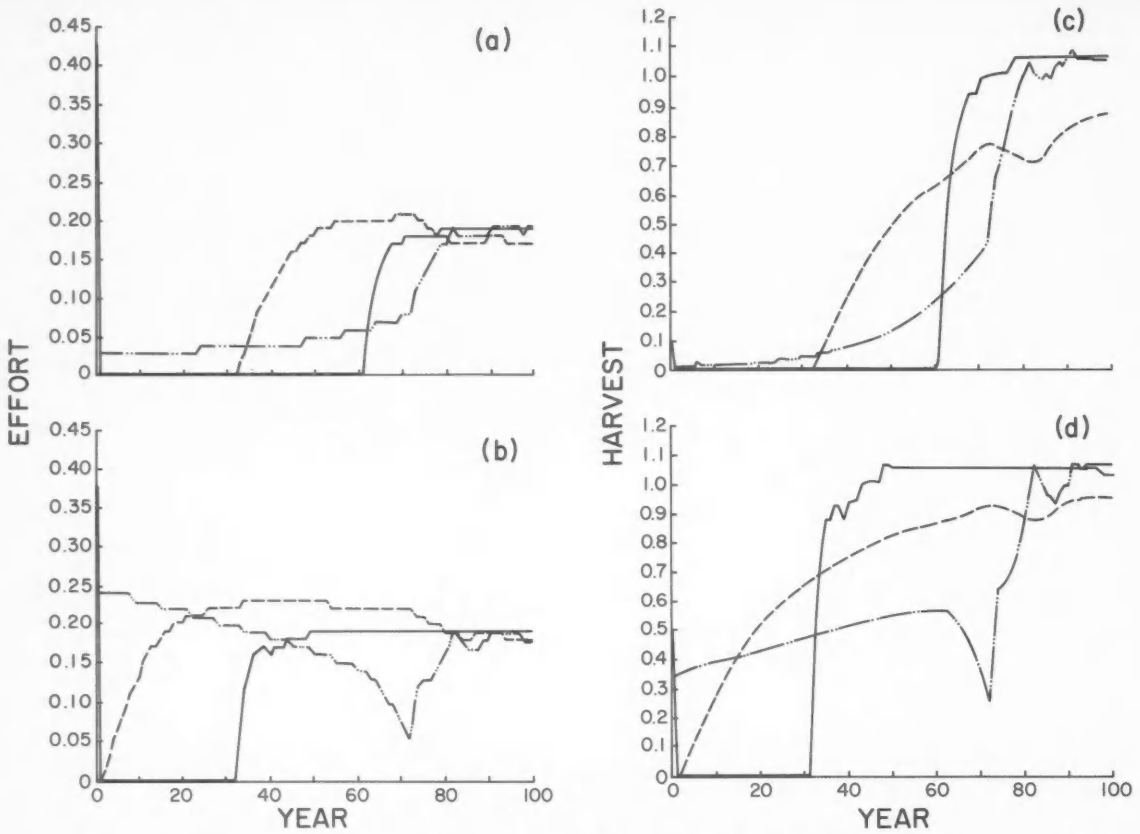

FIG. 3. Optimal levels of fishing effort and harvests for a (a, c) heavily depleted and (b, d) moderately depleted Pacific ocean perch stock, obtained by maximizing harvest (-·-·-), specifying a target spawning stock s^* (—), or specifying a target harvest h^* (– –).

would be negligible if policies were updated regularly.

Variability in recruitment appears to be a significant feature of most commercially important fish stocks (Swartzman et al. 1983); therefore, it is reasonable to question whether the deterministic model used in our analysis is capable of providing useful information to the fishery manager. A stochastic model clearly would be preferable on theoretical grounds; however, in practice, policies obtained from stochastic and deterministic models may be similar because the relative merits of different actions tend to be similar in the two cases (Walters and Hilborn 1978). If a deterministic model is used, a more responsive policy could be obtained by updating the analysis annually. This updated policy would reflect any change in the status of the stock, whether due to random environmental factors or to a change in the level of fishing effort. Another practical advantage of periodic updating is that it can correct errors in previous harvesting policies (Ludwig 1981).

To illustrate this approach, we obtained annually updated policies for achieving a target harvest from the moderately depleted anchovy and Atlantic menhaden stocks (Fig. 4). In each year of a simulated 25-yr period, we obtained an optimal 25-yr rehabilitation policy for the current number-at-age vector $\mathbf{x}(k)$. We then used $f(0)$ from the newly determined policy to obtain $\mathbf{x}(k + 1)$ from equations (1)–(6). Random variation was introduced by incorporating a multiplicative lognormal error term into the stock–recruitment relationship; consequently, $f(0)$ in year k differed from $f(k)$ obtained in year 0. Thus, each annually updated policy in Fig. 4 represents 25 $f(0)$ values taken from a sequence of 25-yr rehabilitation policies. The two updated policies for the anchovy stock do not differ substantially from the policy obtained in year 0; however, the estimated variance (0.06) of the stock–recruitment relationship for this stock was relatively low (Hightower and Grossman 1985). A more pronounced divergence between original and updated policies can be seen for Atlantic menhaden, for which the estimated variance of the stock–recruitment relationship (0.25) was somewhat greater (Hightower and Grossman 1985). Note that effort decreased sharply when strong year classes entered the Atlantic menhaden fishery (Fig. 4b). These decreases were required to obtain harvests closely approximating h^*. It is unclear, however, whether commercial fishermen would be willing to forego increased harvests and accept fluctuations in effort merely to reduce variability in annual harvest. From the manager's viewpoint, the policy obtained in year 0 may be preferred in that changes in fishing effort are more gradual and known in advance; thus, implementation of a given policy would be simpler from both a practical and political standpoint. Finally, it should be noted that information regarding stock condition generally is available only after a lag of several years; hence, annual updates of

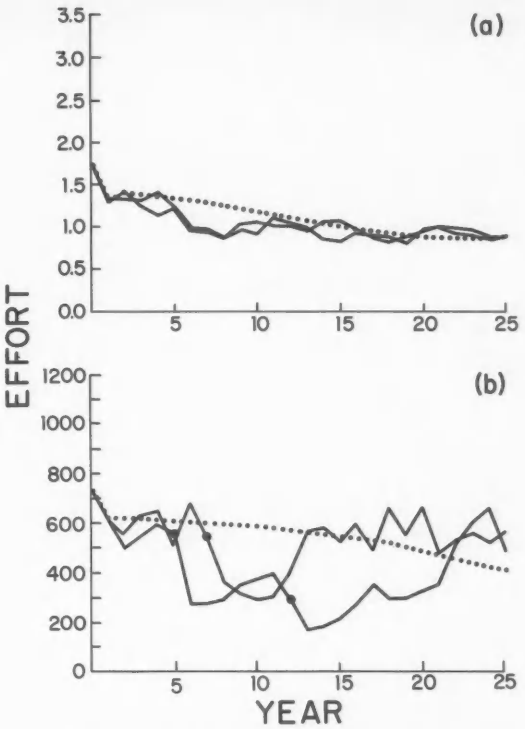

FIG. 4. Optimal (···) and annually updated (—) harvesting policies for moderately depleted (a) anchovy and (b) Atlantic menhaden stocks. For each stock, the three policies were obtained by specifying a target harvest h^*; updated policies were obtained for two simulated 25-yr periods. Closed circles denote years in which a strong year class entered the simulated Atlantic menhaden fishery.

the rehabilitation policy may not be practical.

In cases where variability in recruitment is high and periodic updating is not possible, a stochastic model may be essential. Once a random component is introduced, however, it is no longer meaningful to estimate optimal effort levels for each year of the planning horizon. A better approach would be to develop a stationary policy, where harvest depends only on stock size and not on the number of years remaining in the planning horizon (Ruppert et al. 1985). A stationary policy might have only one (constant effort) or two (egg escapement) parameters (Ruppert et al. 1985) instead of the 25–100 parameters (effort levels) used in our deterministic analysis.

The only obvious disadvantage of using a stochastic model is that much greater computational effort is required to obtain and evaluate optimal policies. An important advantage is that the model can be used to evaluate probabilistic questions that cannot be addressed with a deterministic model. For example, alternative policies could be compared using criteria such as variability in annual harvest (Ruppert et al. 1985), or the probability of full recovery (Overholtz et al. 1986) or of further declines in stock size (Archibald et al. 1983) during the planning horizon. In addition, the probability of stock depletion could be considered explicitly when developing a harvesting policy if a stochastic model was used (Swartzman et al. 1983). The deterministic approach used in this study might play a complementary role in situations where a stochastic model is required by providing insights into the expected behavior of the stock during the rehabilitation period.

In practice, any management plan represents a compromise between user groups with conflicting objectives. These user groups may have widely divergent views regarding the appropriateness of restrictions on fishing effort or of the proper balance between short- and long-term benefits. Consequently, optimization studies might best be viewed as a means to explore and compare policies obtained through the use of alternative management objectives (Peterman et al. 1978). For example, alternative policies for achieving a target harvest can be obtained by varying the length of the planning horizon. A longer planning horizon could be chosen for a stock rehabilitation plan so that changes in the level of fishing effort would be more gradual. As Gunderson (1984) illustrated, management policies requiring rapid changes in either effort or catch levels are difficult to implement. Hence, longer-term planning may be necessary, so that fishermen can attempt to offset reductions in catch by redirecting effort toward less heavily exploited stocks.

Conclusions

Our results for these hypothetical fisheries were, in a general sense, predictable in that an increase in life span or degree of prior exploitation increased the time required for rehabilitation. It appears that rehabilitation will be particularly difficult for long-lived fishes such as Pacific ocean perch, because changes in stock size will be slow and could be masked by variability in recruitment or by errors in the stock assessment. Our results also suggested that the form of the underlying stock–recruitment relationship could affect the rate of rebuilding, in that rehabilitation might be slower if recruitment increased asymptotically than if maximum recruitment occurred at an intermediate stock size. Rehabilitation would also be difficult for stocks with the type of stock–recruitment relationship Overholtz et al. (1986) reported for Georges Bank haddock (*Melanogrammus aeglefinus*). They found that, although recruitment was quite variable, poorer year classes were observed in years when spawning biomass was less than a critical minimum size. In such cases, rebuilding would be particularly slow until the critical minimum size was exceeded.

Although the form of the objective function affected both the rate at which rebuilding occurred and the patterns of annual effort and harvest, mean annual harvest was quite similar for the four cases we considered (Table 2). This suggests that alternative optimal policies might be compared using other criteria, such as the number of years without fishing, or the magnitude of year-to-year changes in effort or harvest. We found that variability in harvest was lowest for the target harvest policy and highest for the target spawning stock and stock biomass policies (Table 2). Variability was high for the latter policies because the fishery remained closed until stock size rebuilt to the target level. Therefore, the best use of those policies may be in determining the minimum time required for full rehabilitation, or alternatively, the maximum time that the fishery should remain closed. Those policies could be practical alternatives in some cases, however, if effort could be diverted to other fisheries during the rehabilitation period. Policies for attaining a target harvest should be somewhat less extreme, although closure might still be optimal for heavily depleted stocks. Closing such a fishery might be a feasible management option, however, because the equilibrium harvest from a

TABLE 2. Mean harvest and coefficient of variation (in parentheses) obtained for each of four objective functions (t = metric tons).

Stock	Historical level of fishing effort	Length of planning horizon (yr)	Objective: Maximize harvest (1000 t)	Objective: Target spawning stock (1000 t)	Objective: Target stock biomass (1000 t)	Objective: Target harvest (1000 t)
Anchovy	f_{max}	25	74.4 (75)	72.7 (80)	72.9 (79)	72.6 (68)
		50	97.9 (47)	95.7 (49)	95.8 (49)	95.7 (43)
	$2 \times f_{0.1}$	25	108.8 (7)	109.8 (28)	109.9 (28)	110.2 (7)
		50	116.9 (12)	114.2 (19)	114.3 (19)	111.1 (13)
Atlantic menhaden	f_{max}	25	433.8 (44)	435.9 (45)	435.9 (45)	427.6 (36)
		50	483.0 (30)	477.7 (30)	477.7 (30)	473.9 (25)
	$2 \times f_{0.1}$	25	513.3 (13)	500.6 (21)	500.5 (21)	500.8 (6)
		50	522.2 (7)	510.0 (14)	510.0 (14)	510.6 (4)
Pacific ocean perch	f_{max}	100	0.4 (113)	0.4 (127)	0.4 (129)	0.4 (82)
	$2 \times f_{0.1}$	100	0.6 (39)	0.7 (68)	0.7 (69)	0.7 (35)

heavily depleted stock may be small (Fig. 1c, 2c, and 3c). If alternative fisheries are not available, policies for maximizing harvest may be more appropriate in that closure would rarely be optimal.

Another approach that would yield less drastic changes in effort would be to rebuild the stock in stages, beginning with a lower target stock biomass or spawning stock. For example, b^* could be the equilibrium stock biomass at f_{msy}, so it would be reached more quickly than the larger stock at $f_{0.1}$. It is likely, however, that no single objective function will provide a directly implementable harvesting policy. Nevertheless, our approach still provides a means of exploring the effects of various management options on stock dynamics and of comparing alternative policies that might be combined into a workable suboptimal policy.

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Density and Size Estimates of Cisco (*Coregonus artedii*) Using Analysis of Echo Peak PDF from a Single-Transducer Sonar

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We estimated size and density of fish in three Wisconsin lakes from echo peak probability density functions (PDFs) obtained at night with a single-transducer 70-kHz echosounder. At night, cisco (*Coregonus artedii*) dominated the pelagic zone in all three lakes. The beam pattern effect was removed with a deconvolving filter technique. Fish size was estimated by fitting a combination of Rice PDFs to the deconvolved fish scattering PDF. Vertical density profiles and size estimates obtained acoustically corresponded to distributions and lengths of fish caught in vertical gill nets. The proportion of different size classes caught in gill nets agreed fairly well with the proportions determined acoustically. This analysis can be applied to signals from noncalibrated sonars and can be used to calibrate simultaneously obtained echo squared integration values. With calibrated sonars, target strength can be estimated in situ. For cisco, TS = 21.9 $\log_{10} L - 67.2$, where TS is target strength in (decibels) and L is fish length (centimetres). The average number of cisco in the three lakes ranged from 89 to 1551 fish/ha, corresponding to a biomass of 2–223 kg/ha. Maximum densities range from 12 to 49 fish/1000 m^3.

À partir de pics d'échosondage (transducteur simple de 70 kHz) effectué la nuit, les auteurs ont déterminé la taille et la densité des groupements de poissons dans trois lacs du Wisconsin à l'aide de fonctions de probabilités de densités (FPD). Au cours de la nuit, le cisco (*Coregonus artedii*) est la principale espèce qui peuple la zone pélagique des trois lacs. On a éliminé l'effet du faisceau à l'aide d'une technique de déconvolution. La taille d'un poisson a été déterminée par ajustement d'une combinaison de FPD de Rice avec un FPD, ayant fait l'objet d'une déconvolution, des dispersions de poissons. Des profils de la densité verticale et des estimations de la taille obtenus par relevés acoustiques correspondaient aux distributions et longueurs des poissons capturés dans des filets maillants verticaux. La proportion des différentes classes de tailles capturées dans les filets maillants était en accord assez élevé avec les proportions déterminées par relevés acoustiques. La présente analyse peut être appliquée aux signaux de sonars non étalonnés et peut aussi être utilisée pour l'étalonnage de valeurs d'intégration de l'écho au carré obtenues simultanément. Avec des sonars étalonnés, on peut déterminer l'importance de la cible *in situ*. Pour ce qui est du cisco, TS = 21,9 $\log_{10} L - 67,2$, où TS est l'importance de la cible (décibels) et L est la longueur du poisson (centimètres). Le nombre moyen de ciscos dans les trois lacs allait de 89 à 1 551 poissons/ha, ce qui correspond à une biomasse de 2 à 223 kg/ha. Les densités maximums variaient de 12 à 49 poissons/1 000 m^3.

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Hydroacoustic or sonar surveys remotely sense the size, abundance, and spatial distributions of fish (see reviews by Thorne 1983; Johannesson and Mitson 1983). These techniques have developed from qualitative to quantitative methods. Echo squared integration is an established procedure, but requires knowledge of the target strength or equivalent back-scattering cross section of the fish. Because the size of fish and exact sonar calibrations are not always known, and because target strength also depends on fish orientation and behavior (Foote 1980, 1981), we need in situ techniques for target strength estimation.

During the last decade, in situ methods have been the subject of much research and development (Ehrenberg 1983a). As a first step, Craig and Forbes (1969) showed that the beam pattern introduces a bias to fish echo amplitudes and must be corrected for or removed. A further development is the dual-beam method (Ehrenberg 1972), which uses simultaneous observations of echoes in the broad and narrow beams to remove the beam pattern effect and estimate target strength. An advantage of the dual-beam method is that the target strength of single fish can be measured. The disadvantage is that a special dual-beam sonar system is required.

Peterson et al. (1976) introduced a single-beam technique using common single-transducer sonars. Originally their tech-

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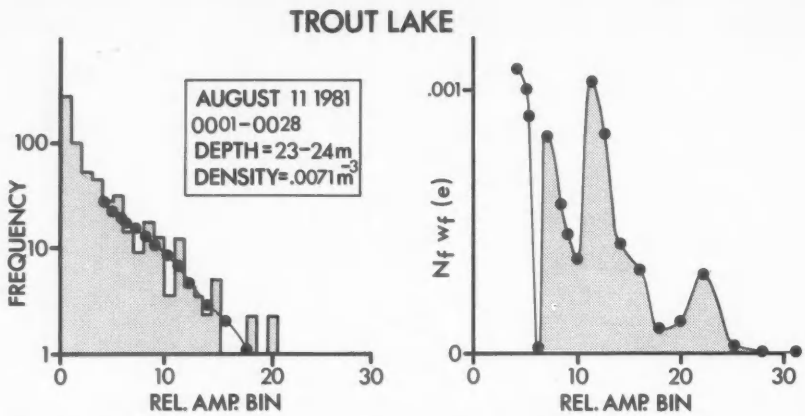

FIG. 1. Distribution of echo peaks (left panel) and the corresponding deconvolved function (right panel). The ordinate of the deconvolved function represents the product of fish density (N_f) and the fish scattering PDF ($w_F(e)$). The units of $N_f w_F(e)$ are per cubic metre per volt. The abscissa is in relative amplitudes. The density N_f is obtained by summing $N_f w_F(e)$ from relative amplitude 5 to 30 (see text). Lower amplitudes represent noise and possible smaller targets.

nique was parametric; they assumed a probability density function (PDF) for the amplitude signals scattered by the fish and then included the beam pattern effect. Derivations and details are given in Clay and Medwin (1977, chap. 7). Clay (1983) obtained the frequency distribution of echo amplitudes by using signal theory and linear filter techniques to deconvolve the effect of the transducer beam pattern. The single-transducer technique requires a good-quality sonar and recording system. The data can be processed on a computer by replaying it in the laboratory. Alternatively, shipboard, real-time processing can be done with a microcomputer-controlled processor.

In this paper, we use the deconvolution technique to estimate relative size distributions and vertical density profiles of pelagic fish in three lakes. These results can be obtained without sonar calibrations. With a calibrated sonar, the method yields in situ estimates of average back-scattering cross sections or target strengths that can be converted to fish lengths. The acoustical results are compared with catches in gill nets. When fish lengths are known, for example from gillnet catches, the relationship of target strength to fish size can be calculated.

Our acoustical analysis requires that most of the echoes come from individual fish. At night, cisco (*Coregonus artedii*) dominated the gillnet catches in our study lakes. Cisco are dispersed across these lakes at night (Rudstam and Magnuson 1985) resulting in a predominance of echoes from single fish.

Theoretical Background

This section is based on previous work by Clay and coworkers (Clay and Medwin 1977, chap. 7; Clay 1983; Clay and Heist 1984). For further details, consult these references and Stanton and Clay (1986).

The peak of the echo envelope must be measured when fish density is low enough to produce single, nonoverlapping echoes. The resulting PDF is the product of two independent processes: 1) the back-scattering at the fish and (2) the location of the fish in the sonar beam. The echo peak PDF is

$$(1) \quad w_E(e) = \int_0^1 w_T(b)\, w_F\left(\frac{e}{b}\right)\frac{db}{b}$$

where e is the echo amplitude, $w_E(e)$ is the PDF of the echo peak (e), $w_T(b)$ is the PDF for observing the amplitude b for a unit scatterer in the transducer beam, and $w_F(e/b)$ is the PDF of the scattering process at the fish. The effect of the transducer directivity pattern can be removed by converting this integral to the usual form of a convolution integral through variable transformations and by subsequent deconvolution. The convolution integral can be solved numerically. An outline of the procedure is given in Appendix A.

The deconvolution operation on the data is equivalent to a linear filter operation. We tested the method on tape-recorded data from different sonars and from lakes with different fish densities. The deconvolution operation was simple to apply and robust. Other methods for removing the transducer directivity use least squares procedures and are described in Craig and Forbes (1969), Ehrenberg (1972, 1983b), and Robinson (1983).

The number of resolved fish echoes is proportional to the number of fish within the insonified volume V_G (gated between the ranges R_1 and R_2, see Fig. 3). The first processing step determines the frequency $f_E(e)$ of echo amplitudes within each amplitude bin ($e \pm \Delta e/2$); $f_E(e)$ is proportional to the echo peak PDF $w_E(e)$, fish density N_f, number of pings M_p, the volume V_G, and Δe:

$$(2) \quad f_E(e) = N_f M_p V_G w_E(e) \Delta e$$

where $V_G = 2\pi/3\,(R_2^3 - R_1^3)$, $f_E(e)$ is measured, the product $M_p V_G \Delta e$ is known, and N_f is unknown. Since $f_E(e)$ is proportional to $w_E(e)$, we can use $f_E(e)$ for $w_E(e)$ in Eq. (1). Our processor gives $f_E(e)$ on a linear amplitude scale. The $f_E(e)$ were resampled on a logarithmic scale because the deconvolution algorithm uses a logarithmic amplitude scale. We deconvolve $f_E(\log e)$ using $w_T(b)$ (Appendix A). The resulting numbers (transformed back to a linear scale for display; by analogy denoted $f_F(e)$) are proportional to fish density (N_f) and $w_F(e)$:

$$(3) \quad N_f w_F(e) = f_F(e) / (V_G M_p \Delta e).$$

After deconvolution, we obtain values for $N_f w_F(e)$ at discrete values of e (Fig. 1). Since the integral of $w_F(e)$ over all

TABLE 1. Limnological characteristics of the lakes and density estimates of pelagic fish (cisco) from acoustics and gill nets. Only the deepest basin in Trout lake is included. (The total area of Trout Lake is 1605 ha.) Several measures of fish abundance are given. The maximum density is the highest value recorded acoustically within a 1-m depth stratum. The number of fish in each lake is calculated as the average fish density at a depth stratum multiplied by lake volume at that depth. This value is used to obtain the average number of fish per hectare. The biomass is calculated by multiplying with the average weight of cisco caught in gill nets (corrected for net selectivity).

Lake	Trout	Big Muskellunge	Mendota
Limnology			
Area (ha)	770	396	3983
Maximum depth (m)	36	21	25
Mean depth (m)	18	7	12
Alkalinity (mmol/L)	0.82	0.39	1.40
pH	8.3	8.2	8.7
Fish abundance			
Maximum density (no./1000 m^3)	49	12	32
Fish in lake	1 190 000	35 000	2 040 000
Fish/ha	1 551	89	515
Biomass (kg/ha)	51	2.4	223
CPUE[a]	71	96	70
CPUE, corrected[b]	123	214	62
CPUE[b]/(fish/ha)	0.08	2.4	0.12

[a]Catch in seven nets set for 24 h.
[b]Catch corrected for net selectivity.

e is 1, a summation approximation is also 1 and we can calculate N_f:

$$(4) \quad \sum_i N_f w_F(e_i)\Delta e_i = N_f \sum_i N_f w_F(e_i)\Delta e_i = N_f.$$

In practice, the lower summation limit is chosen so as to exclude noise and possible smaller targets (fish larvae, larger zooplankton, etc). We use the shape of the deconvolved function to determine this lower summation limit (see Fig. 1). The calculation of N_f does not require insertion of sonar calibrations (see Clay and Medwin 1977, p. 247).

A fish is a complicated scatterer; one needs an ensemble of echoes to describe a single fish (Ehrenberg et al. 1981; Clay and Heist 1984). On the basis of signal theory, Clay and Heist chose the two-parameter Rice PDF to describe the fish scattering PDF ($w_F(e)$) (Fig. 2; Appendix B). They identified two components of the back-scattering cross section, one a constant component σ_c describing the repeated nonfluctuating component of echo amplitudes and the other a fluctuating component, σ_d. The back-scattering cross section σ_{bs} is

$$(5) \quad \sigma_{bs} \equiv \sigma_d + \sigma_c \qquad \gamma \equiv \frac{\sigma_c}{\sigma_d}$$

where σ_{bs} and γ are used in the two-parameter Rice PDF. We attribute σ_c to a concentrated scatterer like the swimbladder; σ_d is related to the rest of the fish structure.

There are differences in the literature in the definitions of back-scattering cross section. However, the literature is consistent on the meaning of target strength TS.

We follow Clay and Medwin (1977) and use

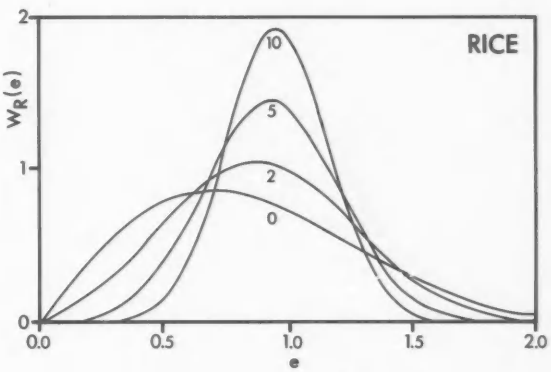

FIG. 2. Rice PDFs with $\sigma_{bs} = 1$ and different values of γ.

$$(6) \quad TS \equiv 10 \log_{10} \sigma_{bs}.$$

Others (e.g. Johannesson and Mitson 1983) use

$$(7) \quad TS \equiv 10 \log_{10} \left(\frac{\sigma}{4\pi}\right) \qquad \sigma \equiv 4\pi\, \sigma_{bs}$$

where the nonsubscripted σ represents the total scattering cross section and σ_{bs} represents the scattering of sound in a particular direction.

Love (1977) and Foote (1979) provided empirical equations relating σ_{bs} to fish length. Translated to our notation, Love's equation is

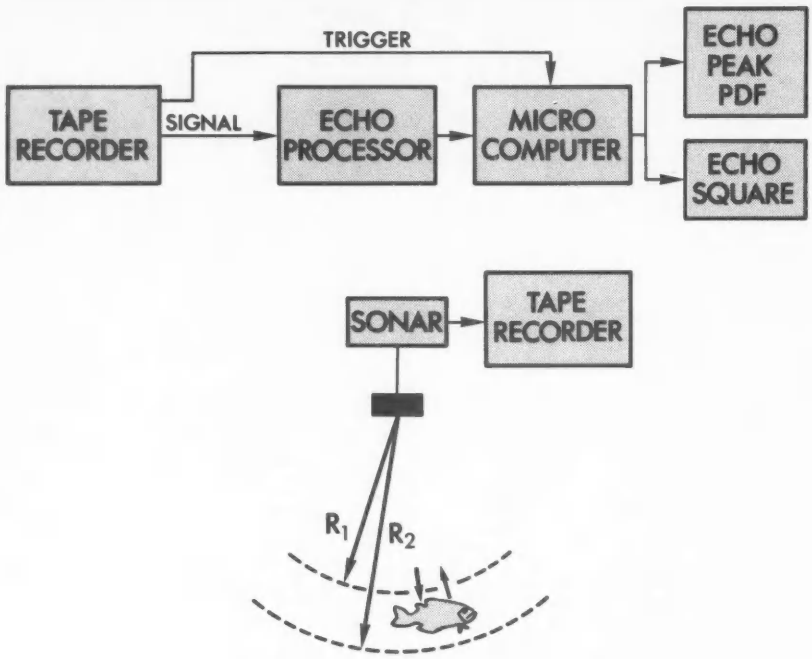

FIG. 3. Data collection and processing.

$$(8) \quad 4\pi\,\sigma_{bs} = a\left(\frac{L}{\lambda}\right)^{b}\lambda^{2}$$

where L is fish length, λ is acoustic wave length, and a and b are empirical constants. Love (1977) fitted this equation to measurements of length and back-scattering cross sections from many fish species and obtained dorsal aspect values for a and b of 0.056 and 1.88, respectively. The scatter about the mean is of the order of a factor of 2. We solved Eq. (8) for L and used estimates of σ_{bs} to compute fish length L:

$$(9) \quad L = \left(\frac{4\pi\sigma_{bs}}{a\lambda^{2}}\right)^{1/b}\lambda$$

where L and λ are in metres.

It is important to recognize that repeated echoes from fish of a given size fluctuate and can have wide or narrow frequency distributions of echo amplitudes. γ depends on fish behavior and the ratio of fish length to wave length (L/λ). When L/λ is small and the fish quiet, γ becomes large. For large active fish, γ tends to 0 and the Rice PDF approaches the previously used Rayleigh PDF (Clay and Heist 1984).

Study Area

Trout and Big Muskellunge lakes are located in Vilas County in Wisconsin's Northern Highland Lake District. They are surrounded by conifer and aspen–birch forests and are within 5 km of the Trout Lake Station of the University of Wisconsin-Madison (Magnuson and Frost 1982). Lake Mendota, located in Dane County, southcentral Wisconsin, is in a predominantly agricultural and urban area. Morphometric and limnological characteristics are summarized in Table 1.

Materials and Methods

The lakes were sampled between July 31 and August 24, 1981, using seven vertical gill nets, each with a different mesh size (19-, 32-, 38-, 51-, 64-, 89-, and 127-mm stretch mesh). Details on the construction of the nets and on the sampling procedure are given in Rudstam and Magnuson (1985). The proportions of different cisco age groups were corrected by taking into account both the size selectivity of the mesh and the higher encounter probability for larger fish (Rudstam et al. 1984).

The five lakes were surveyed at night with a 70-kHz echo sounder (Simrad EY-M, 70-24-F transducer, 11° beam width between half power points). The transducer was towed about 0.5 m below the surface from an "A-frame" in front of a small boat. Towing speed was approximately 1.5–2 m/s. The Simrad EY-M Sonar gives an output signal in the 10-kHz audio frequency range and this signal was recorded on casette tapes.

Sonar tapes were analyzed in the laboratory using a microcomputer-based digital echo processor described by Powell and Stanton (1983). A diagram of data collection and analysis procedure is presented in Fig. 3. Time gates were chosen to yield integrated echoes and distributions of echo peaks for each 1-m depth interval. The transducer directivity was removed with the deconvolving technique (Appendix A). Our processor gives the echo peak as the maximum value of the echo envelope achieved within a time gate (see Stanton and Clay 1986). The tail of an echo that straddles two adjacent time gates may therefore contribute a second smaller amplitude to the echo peak PDF if there is no other larger fish echoes within the time gate. This could result in an overestimation of small echoes, but the effect is not large enough to distort our deconvolved fish scattering PDFs (see Fig. 7). A 1-m depth gate was chosen as

a compromise between the need to separate single fish echoes and the problem of echoes straddling two depth intervals.

Since larger sample sizes give more accurate echo distributions, we combined several depth intervals from both transects for the analysis of fish lengths (see Fig. 7). Fish size was estimated by fitting a linear combination of Rice PDFs to the deconvolved data points.

Results and Discussion

Abundance and Distribution

Cisco and yellow perch (*Perca flavescens*) constituted over 95% of all fish caught in the gill nets. As cisco was the only species caught at night, we assumed that all targets from night time sonar transects were cisco. In Big Muskellunge Lake, however, we also observed many small young-of-the-year (YOY) yellow perch (about 3–4 cm, too small for the mesh sizes used) in the open water at night. Larger perch were caught from dawn to dusk and were spatially segregated from cisco in Trout and Big Muskellunge lakes, but not in Lake Mendota (Rudstam and Magnuson 1985). The size and number of different cisco age groups are presented in Table 2. In Trout Lake, we treated the two depth intervals 4–24 and 24–32 m separately, since no cisco smaller than 19 cm total length (mainly YOY and 1+) were caught below 24 m depth. There was no difference in depth distribution in the gill nets of different cisco age groups in Big Muskellunge Lake. In Lake Mendota, only larger cisco were caught.

Density and size estimates from echo peak PDF are valid only at depths where single echoes predominate. If many echoes are overlapping, densities will be underestimated. However, echo squared integration remains proportional to true densities (Foote 1983). If the fish size distribution can be assumed to be constant, deviations from a linear regression of echo squared integration on echo peak density will indicate the presence of many overlapping echoes. This does not occur in the lakes investigated here (Fig. 4). As expected from theory, the slope of this regression varies with average fish size and is highly correlated with average length squared of fish caught in gill nets ($r = 0.97$, $N = 4$, $P < 0.01$). The correlations between densities and echo squared integration at low fish densities can be used to calibrate echo integration values. This allows density to be estimated also in areas with overlapping echoes when single and overlapping echoes are from the same fish population.

The vertical distributions obtained acoustically corresponded well with catches from gill nets (Fig. 5 and 6). The fish occupied a restricted depth interval, partly as a response to temperature and low oxygen levels (Rudstam and Magnuson 1985). In Trout Lake, the whole water column was only sampled with the 32- and 38-mm-mesh nets, but these nets caught 89% of all cisco. The general feature of the vertical distribution — a shallow and a deep peak — is apparent in both net and acoustic results. The number of fish caught in deep water as compared with shallow water is larger than the comparable relative densities determined acoustically. Corrections for net selectivity account for only part of this difference. Cisco occurred in deep water in Trout Lake during both day and night but in shallow water only during the night (Rudstam 1983). Therefore, because the netting time included morning hours, fish were available in deep water for a longer time period than in shallow water. Also, net visibility may decrease with depth and

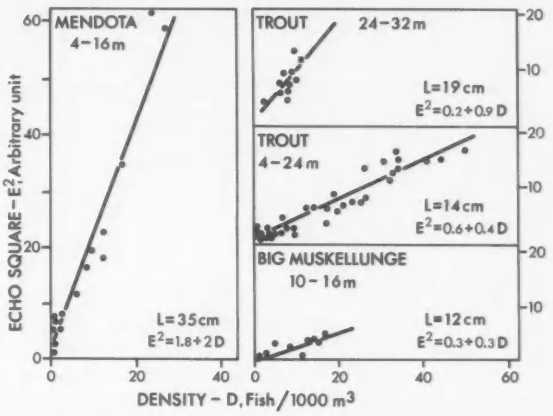

FIG. 4. Linear regression of echo squared integration and fish densities calculated from echo peak PDF from Lake Mendota, Big Muskellunge Lake, and two depth intervals, 4–24 and 24–32 m, in Trout Lake. L = average fish lengths from gillnet catches.

result in an increased catch probability in deeper water. Neither 1+ nor older cisco were caught shallower than 10 m, corresponding with acoustical results.

In Big Muskellunge Lake, the lower limit of the cisco distribution, determined acoustically and with nets, coincides. The upper acoustical limit is obscured by abundant small scatterers. These targets may be YOY yellow perch which were common in the pelagic region during the night (L. G. Rudstam, pers. obs.). Because of overlapping echoes, analysis of echo peak PDF cannot be used to calculate densities of these smaller targets visible as a peak in echo square integration values between 6 and 7 m depth. However, a lower bound for their density (190 fish/1000 m^3 between 6 and 7 m depth) can be calculated using geometric arguments (see Stanton 1985).

In Lake Mendota, the vertical distributions are more restricted in the acoustical profiles than in the nets. The sonar chart revealed that the gill nets were set where the vertical distribution of targets was more spread out than in the rest of the transect (Fig. 5). Based on temperature preference and oxygen avoidance reactions, we had previously predicted a more restricted vertical distribution of cisco than observed in gill nets (Rudstam and Magnuson 1985).

Although the vertical distributions obtained with nets and acoustics compare favorably within each lake, comparisons of acoustic density estimates and catch per unit effort (CPUE) among lakes do not. The ratio of CPUE to the number of fish per hectare is much higher in Big Muskellunge Lake than in the other two lakes (Table 1). The reason is unknown. Sonar charts clearly show lower densities of targets in Big Muskellunge compared with the other two lakes (Fig. 5). The catches in Big Muskellunge Lake in 1982 and 1983 using the same effort were very low (14 and 0 cisco, respectively) and it is therefore unlikely that the population was large in 1981. The nets may have been located in an area where cisco aggregate, although this is not apparent on sonar charts. These results demonstrate the problems associated with using data from one sampling gear set at one location for comparisons among lakes.

The number of cisco in the Wisconsin lakes (89–1551 fish/ha) is comparable with the number of vendace (*Coregonus albula*) obtained acoustically in a Finnish lake (530 fish/ha

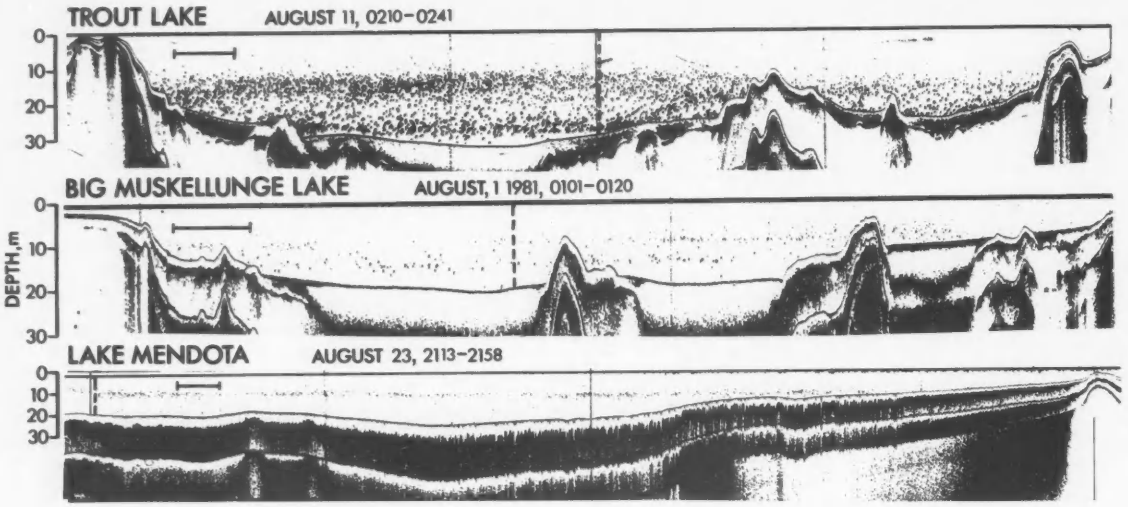

FIG. 5. Sonar charts from the transect in each lake. The locations of gill nets are indicated with broken lines. Scale represents 200 m.

TABLE 2. Characteristics of the cisco populations in the three lakes (calculated using all fish caught in gill nets) and the median depth of cisco caught in the nets at night.

Fish group	$\bar{x}$ length (mm)	SD	$\bar{x}$ weight (g)	Median depth (25–75%)	N^a	N^b
Trout Lake						
YOY	9.8	0.4	7	14 (13–20)	19	12
1+	15.0	0.5	24	16 (14–19)	55	32
Older	20.1	1.0	56	26 (23–27)	102	35
Big Muskellunge Lake						
YOY	9.8	0.4	8	10 (10–11)	119	74
1+	18.7	0.7	55	10 (9–12)	61	47
Older	24.7	1.7	136	9 (9–12)	6	3
Lake Mendota						
Older	34.6	1.3	433	6 (4–8)	139	64

[a]Total number of fish caught.
[b]Number of fish caught at night.

in August, Jurvelius et al. 1984). The high densities in Trout Lake can be partly attributed to a concentration of cisco in the deep basin during the summer. The other basins, constituting 48% of the total surface area, have less volume of water with a lower temperature and high oxygen levels (Robertson 1984). Fewer targets are observed in these basins (L. G. Rudstam, pers. obs.).

Fish Length

Deconvolved fish scattering PDFs ($w_F(e)$) are presented in Fig. 7. A given size fish gives rise to a distribution of echoes that can be adequately described with a Rice PDF (Clay and Heist 1984). The data in Fig. 7 show the presence of at least two groups of fish in each depth interval, and a linear combination of two or three Rice PDFs were therefore fitted to the deconvolved fish echo PDFs. This yields estimates of the scattering parameters σ_{bs} and γ (Table 3). In contrast with other published comparisons of target strength distribution and fish size (e.g. Lindem 1983; Dickie et al. 1983), this approach has the advantage of accounting for the whole distribution of echo peaks expected from a size group of fish.

Estimations of fish length from in situ measurements of σ_{bs} require insertion of sonar system calibration. The system calibration is a number that only depends on the gain settings and fixed system parameters. Since σ_{bs} from the fit to the Rice PDF is the back-scattering cross section (square metres), we can calculate fish length using Eq. (9) with Love's (1977) estimation of the constants a and b (0.056 and 1.88). Figure 8 shows acoustic estimates of fish lengths compared with measurements of fish caught in gill nets. Evaluations are for 70 kHz and sound velocity 1470 m/s. The 45° line indicates a perfect match and the data are evenly distributed about the line. The fit is very good.

We did a least squares fit of acoustic and gillnet data to get estimates of the Love parameters for cisco and obtained $a = 0.027$ and $b = 2.19$ ($r = 0.99$, $N = 11$, $P < 0.01$). The difference between these and Love's values is within the range of measurements presented by Love. The corresponding target strength equation is

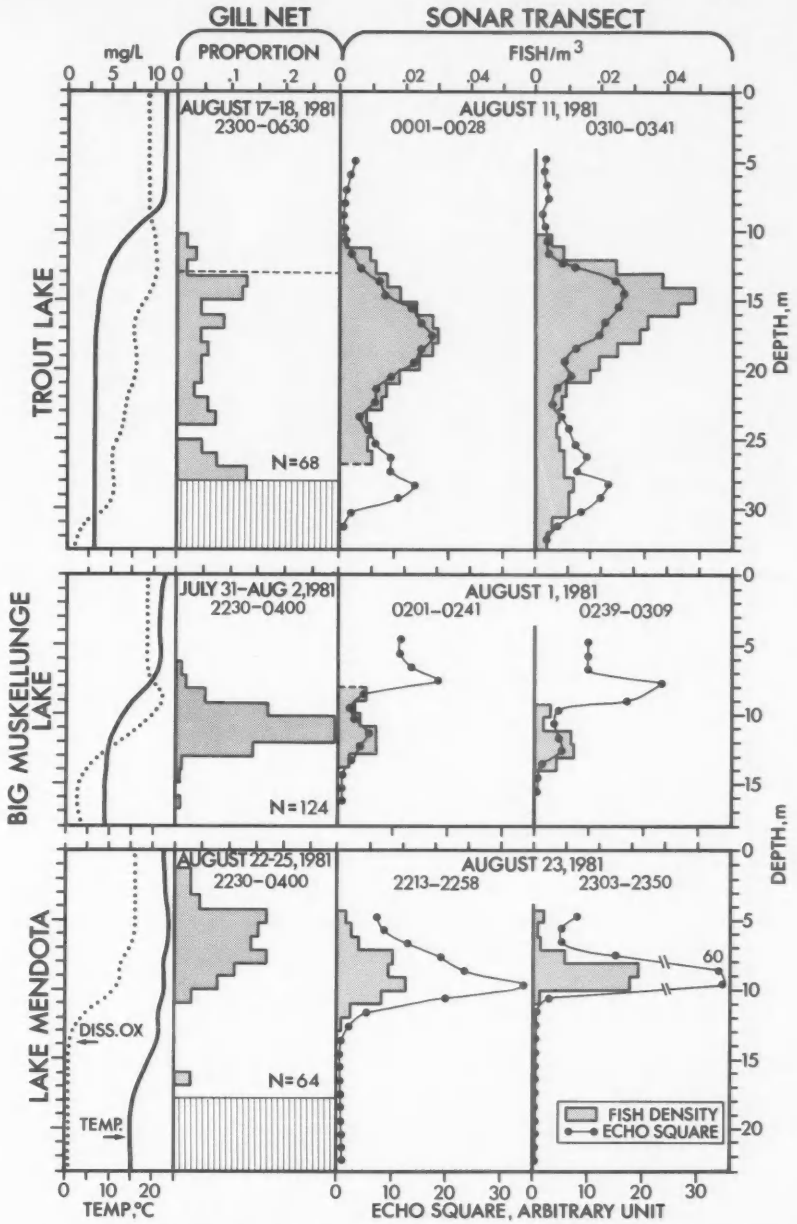

FIG. 6. Depth distribution of cisco obtained with gill nets and with acoustics (analysis of echo peak PDF and echo integration), and profiles of dissolved oxygen and temperature in the three lakes. Fish densities from echo peak PDF could not be calculated above 8 m depth in Big Muskellunge Lake (overlapping echoes) and below 26 m depth for the first transect in Trout Lake (small sample size). The differences between echo integration and fish density profiles are due to differences in average fish size among lakes and between depth intervals in Trout Lake (see Fig. 4). Only the 32- and 38-mm-mesh nets were fished above the broken line in Trout Lake (see text).

$$(10)\quad TS = 21.9 \log_{10} L - 67.2$$

where TS is given in decibels and L is given in centimetres. This is similar to an equation presented by Lindem and Sandlund (1984) for a combination of smelt (*Osmerus eperlanus*), vendace, and whitefish (*Coregonus lavaretus*) in Lake Mjøsa, Norway, using the same type of echosounder as in this study. The equation for cisco is also close to equations given

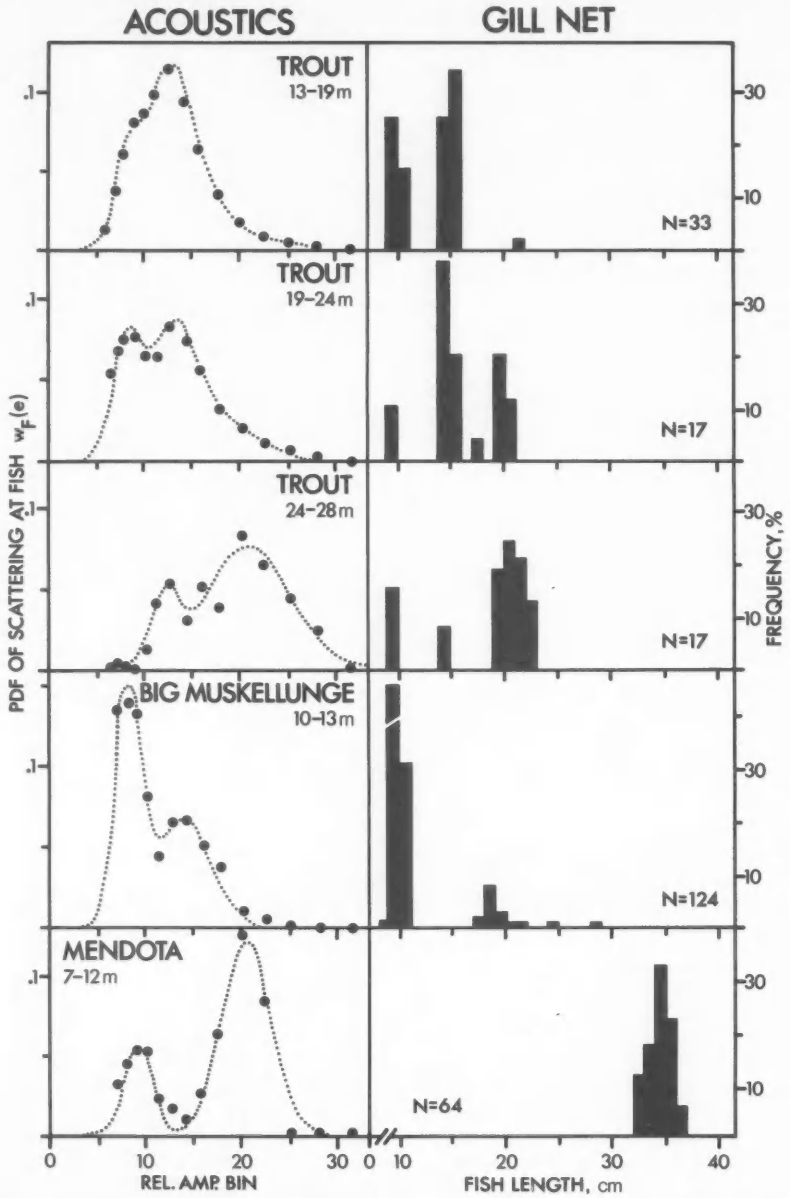

FIG. 7. Deconvolved fish scattering PDF ($w_F(e)$) for selected depth intervals fitted to a linear combination of Rice PDFs, and the size distribution of cisco caught in gill nets. Data from both transects in each lake are combined. The amplitude bins for Lake Mendota are approximately 50% larger than comparable amplitude bins in the other lakes due to different amplifications in the processor. The Rice PDF parameters are summarized in Table 3.

for gadoid fishes (Foote 1979).

The minimum relative difference in fish lengths that can be separated will vary with the lake studied because it depends on species, fish activity, and distinctness of the size classes in the population. In our lakes, the relative length differences between adjacent size groups were approximately 50% (Table 2) and we could separate the contribution from YOY, 1+, and older cisco to the fish scattering PDF ($w_F(e)$). The relative proportion of these age groups could then be calculated. These proportions compare favorably with proportions caught in gill nets in Trout Lake, less so in Big Muskellunge Lake (Table 3). In Lake Mendota, a smaller fish group (15 cm) not caught in gill nets was identified with acoustics. These fish could have been YOY cisco. YOY cisco caught in early September 1982

TABLE 3. Comparison of fish lengths and proportions of different year classes of cisco as determined by nets and acoustics. The proportions in gill nets have been corrected for net selectivity. Parameters of the Rice PDFs fitted to the deconvolved data points and the rms error are also given.

Lake	Age	Length (mm)		Proportions (%)		Rice PDF parameters		
		Net	Acoustics	Net	Acoustics	σ_{bs} (mm^2)	γ	Error (%)
Trout Lake (13–19 m)	YOY	98	87	38	12	29	20	
	1+	150	143	61	76	74	10	2.8
	Older	211	213	1	10	154	10	
Trout Lake (19–24 m)	YOY	95	84	10	24	27	13	
	1+	148	143	56	55	72	10	2.7
	Older	196	214	34	21	153	10	
Trout Lake (24–28 m)	YOY	95	—	15	0	—	—	
	1+	145	131	8	15	61	30	4.3
	Older	208	238	77	85	188	10	
Big Muskellunge	YOY	98	85	83	50	27	15	
	1+	187	154	15	50	85	10	3.4
	Older	247	—	2	0	—	—	
Mendota (7–12 m)	YOY	—	150[a]	0	22[a]	80	15	
	1+	—	—	0	—	—	—	4.3
	Older	346	353	100	78	404	30	

[a]This size group was not caught in the nets. They may represent YOY cisco (see text).

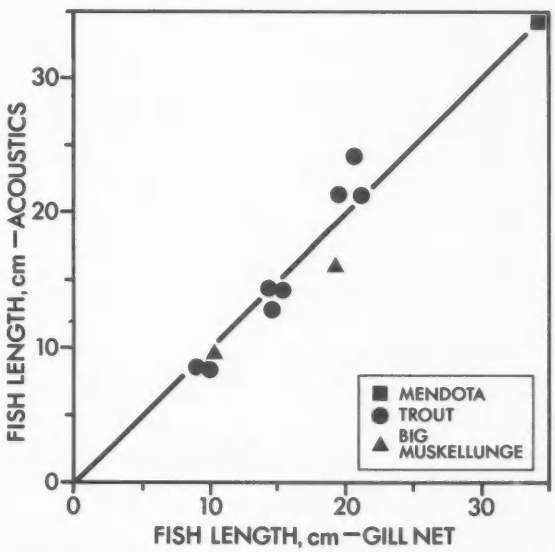

FIG. 8. Acoustic estimates of fish length versus average length of fish caught in gill nets.

were 13 cm long. In 1981, the mesh sizes used had very low selectivity for cisco of that length (Rudstam et al. 1984). Also a few 1-yr-old cisco were caught in 1982, indicating that YOY cisco must have been in the lake the previous year.

The value for γ ranged from 10 to 30 for different cisco sizes and lakes (Table 3). Clay and Heist (1984) reported values of 10–50 for rainbow smelt (*Osmerus mordax*) in Lake Superior. They also observed the dependence of γ on fish activity using the common shiner (*Notropis cornutus*) and found γ to be 33 for very calm fish and 3.5 for fish moving "naturally." The high γ observed for cisco may indicate that this fish is relatively calm at night in our study lakes. Laboratory experiments on other species of the genus *Coregonus* indicate a lower activity at night (*C. lavaretus*, Eriksson and Kubicek 1972; *C. clupeaformis*, Scherer et al. 1986). However, we hesitate to use γ as an index of fish behavior because γ also depends on the distinctness of the size classes and may be species specific.

Evaluation of the Acoustic Method

Acoustical analysis gives a somewhat different picture of fish abundance and spatial distribution than do gill nets. Within the time it takes to do a survey, sonar integrates over space. Gill nets sample a limited area of the lake and integrate over time. A perfect one-to-one correspondence cannot be expected because fish density and fish sizes at the gillnet location will not be exactly representative of the average fish density in the lake. Therefore, a calibration of acoustics with net catches requires distinct size classes and a reasonably uniform size distribution across a lake. It is also necessary to compensate for the selectivity of the nets. Above, we introduced sonar calibrations to estimate the constants a and b in Eq. (9) and obtained a relationship between TS and cisco length (Eq. (10)). In principle, this analysis can be performed on data from noncalibrated, high-quality echo sounders. We could have estimated values for a and b with σ_{bs} measured in relative amplitudes without using sonar calibrations. However, calibrated sonars are preferable, since it is then possible to use acoustic fish length calculations as a guide when matching peaks in the fish scattering PDF with the lengths of sampled fish. Other fish species may give different regressions and a field calibration may have to be undertaken for each system investigated.

Figure 8 is presented as a comparison of acoustic length estimates using Love's equation and length estimates from gill nets. Burczynski and Johnson (1986) recently used Love's equation to calculate fish length from target strength measurements on juvenile sockeye salmon (*Oncorhynchus nerka*)

using a dual-beam sonar. Their acoustic length estimates were close to the lengths of fish caught in trawls. Cisco and sockeye salmon are members of the same family and appear to have similar acoustic properties. Larger deviations between acoustic length estimates using Love's equation and actual fish lengths may be obtained with other fish species.

Portable, single-transducer scientific echo sounders are practical to use for surveys in smaller lakes. Such lakes often have pelagic fish populations that disperse at night, making methods involving the analysis of echo peak PDFs particularly applicable. However, the potential for such methods in large lakes and oceans should not be underestimated. We find the relatively good correspondence within each lake between acoustic and net estimates of vertical distributions, fish sizes, and proportions of cisco age groups to be promising. The present work shows that the use of single-beam sonars can be expanded to address questions involving the size structure within populations, such as habitat partitioning among age or size groups or among different sized fish species, and spatial and temporal dynamics of predator–prey interactions.

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Appendix A

Deconvolution techniques are used in electronics and geophysics to recover an input signal that has been transformed by a linear filter. The technique operates on a convolution integral. With the following substitutions:

$$b = \exp(-x) \qquad 0 < b < 1$$

$$e = e_0 \exp(-y) \qquad 0 < e < e_0$$

$$\frac{db}{b} = -dx,$$

Eq. (1) from the main text becomes

$$(A.1)\quad w_E(e_0 \exp(-y)) = \int_0^\infty w_T(\exp(-x))\, w_F(e_0 \exp(x - y))\, dx.$$

This is a converging integral and has the form of a convolution. The Laplace z-transformation ($z = \exp(-i\alpha)$) of a convolution integral is simple (e.g. see Hildebrand 1965) and can be used for numerical evaluation. Let x and y assume a sequence of values:

$$x_m = m \cdot a \quad \text{(equivalent to } b_m = \exp(-ma)\text{)}$$

$$y_n = n \cdot a \quad \text{(equivalent to } e_n = \exp(-na)\text{)}$$

$$a \equiv \log_e(10)/N$$

for N equal steps per decade where m and n are integers. We change the convolution integral to an equivalent summation and let dx become a. The Laplace z-transformation of the integral then becomes

$$(A.2)\quad W_E(z) = aW_T(z)\, W_F(z)$$

where W denotes the Laplace z-transformation of w. The transforms can be written as polynomials:

$$W(z) = C_0 + C_1 z + C_2 z^2 + \ldots$$

where C represent constants. From Eq. (A.2), we get

$$(A.3)\quad W_F(z) = \frac{W_E(z)}{aW_T(z)}.$$

The values of b_m and e_n are equally spaced on a logarithmic scale because of the transformations. $W_T(z)$ can easily be computed for different b_m (e.g. see Ehrenberg 1972; Peterson et al. 1976; Clay and Medwin 1977). Evaluations of the constants for $W_E(z)$ require resampling the linearly sampled data at equally spaced logarithmic increments. We proceed as follows: (1) the linearly sampled data are gathered into approximate logarithmically spaced amplitude bins, (2) a second-order polynomial is fitted to data over a number of data points, and (3) the correctly spaced e_n are interpolated from this polynomial fit. The result is sensitive to the number of steps per decade (N) and the number of data points used for smoothing. We find that a value of N of 20 steps per decade and smoothing over six data points give good results.

Finally, $W_E(z)$ and $W_T(z)$ are replaced by their polynomial expressions, and algebraic long division or a recursive algorithm of the polynomials gives values for the constants in $W_F(z)$. See Clay (1983) and Stanton and Clay (1986) for further details.

Appendix B

The Rice PDF describes a sine wave plus noise. Clay and Heist (1984) equated the sine wave with a coherent component of the fish scattering process (σ_c) and the noise with a distributed component (σ_d). The ratio of $\sigma_c/\sigma_d = \gamma$ represents the signal to noise ratio of the echo. Using Eq. (5) from the main text, the Rice PDF can be written as

$$w_R(e) = \frac{2e(1+\gamma)}{\sigma_{bs}} \exp\left(-\frac{(1+\gamma)e^2 + \gamma\sigma_{bs}}{\sigma_{bs}}\right) I_0(q)$$

where

$$q \equiv \frac{2e(\gamma(1+\gamma))^{\frac{1}{2}}}{\sigma_{bs}^{\frac{1}{2}}},$$

e is echo amplitude, and I_0 is the modified Bessel function.

Electrophoretic Variation, Regional Differences, and Gene Flow in the Coho Salmon (*Oncorhynchus kisutch*) of Southern British Columbia

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Wehrhahn, C. F., and R. Powell. 1987. Electrophoretic variation, regional differences, and gene flow in the coho salmon (*Oncorhynchus kisutch*) of southern British Columbia. Can. J. Fish. Aquat. Sci. 44: 822–831.

Populations of coho salmon (*Oncorhynchus kisutch*) in southern British Columbia were assayed for genetic variation at 26 enzyme loci. The mean heterozygosity was only 0.25 ± 0.06%. Previously studied wild Oregon cohos had a mean heterozygosity of 1.36 ± 0.37% compared with 0.30 ± 0.09% in Lower Coastal Mainland and 0.13 ± 0.08% in Upper Fraser River fish for the same enzyme loci. A plausible explanation for the very low mean heterozygosity is that British Columbia coho salmon went through an extreme population bottleneck during or after the last ice age. Lower Coastal Mainland salmon are significantly different from the fish of Vancouver Island and can be easily distinguished from Oregon and Capilano Hatchery (Coastal Mainland, B.C.) fish. It should be feasible to determine the relative proportions of different stocks in large oceanic coho salmon samples. The maximum likelihood estimate of the migration rate between spawning populations is 5.8 ± 1.2 breeding adults per generation. This is enough to prevent adaptation to local habitats by small populations of fewer than 100 breeding adults, but it is not high enough to impede selection in large populations of 1000 or more breeding adults.

Les auteurs ont analysé la variation génétique pour 26 locus enzymatiques chez des populations de saumon coho (*Oncorhynchus kisutch*) peuplant la partie sud de la Colombie-Britannique. L'hétérozygosité moyenne ne s'élevait qu'à 0,25 ± 0,06 %. Chez des cohos sauvages de l'Orégon ayant fait l'objet d'une étude antérieure, l'hétérozygosité moyenne avec les mêmes locus se chiffrait à 1,36 ± 0,37 % contre 0,30 ± 0,09 % chez les cohos de la région de Vancouver et 0,13 ± 0,08 % chez ceux du haut Fraser. Il est fort possible qu'au cours de la dernière période glaciaire ou après, le saumon coho de la Colombie-Britannique ait été l'objet d'un encombrement démographique marqué qui expliquerait la très faible hétérozygosité moyenne. Le saumon de la région de Vancouver est nettement différent de celui de l'île Vancouver et peut être facilement différencié des poissons de l'Orégon et de la piscifacture de Capilano (région côtière continentale). Il devrait être possible de déterminer les proportions relatives des différents stocks chez d'importants échantillons de saumons cohos capturés en mer. L'évaluation de la vraisemblance maximum du taux de migration entre les populations reproductrices s'élève à 5,8 ± 1,2 géniteurs par génération. Ceci suffit à empêcher l'adaptation aux habitats locaux dans le cas de petites populations totalisant moins de 100 géniteurs mais il n'est pas assez élevé pour entraver la sélection chez de grandes populations de 1 000 géniteurs ou plus.

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The extent to which coho salmon (*Oncorhynchus kisutch*) occur as ecologically and geographically differentiated stocks in southern British Columbia is a matter of practical interest to fisheries managers, particularly when large-scale salmon enhancement projects are contemplated. The discovery of distinct genetic populations within the area would establish the need for stock conservation during enhancement and possibly provide a tool for stock identification as well. Conversely, if regional populations cannot be distinguished by genetic methods, there might be less need for managers to take population structure into account.

Utter et al. (1973a) reported the first extensive survey of electrophoretic variation in coho salmon. They found three polymorphic lock, *Ldh-4* (two alleles), *Pgm-1* (two alleles), and transferrin (three alleles), among the 23 loci they surveyed in 971 fish. The mean heterozygosity was 1.8%, ranging from 0.5% in Columbia River coho ($N = 347$) to 2.7% in Washington coast coho ($N = 248$). The transferrin B allele was present in 10–35% of coho from the Washington coast including Puget Sound, but was absent in the Columbia River, whereas the transferrin A allele was more common in the Columbia River (90%) than on the Washington coast and Puget Sound (14.9–37.3%). The distinct transferrin frequencies suggest that geographically separated stocks such as those from the Columbia River and Washington coast are to a large degree reproductively isolated.

Coho salmon occupy the greatest diversity of habitats among the five species of Pacific salmon, including many streams not suitable for its congeners (Aro and Shepard 1967). The abundance of small streams with suitable coho spawning habitat should allow extensive gene flow among nearby streams within regions. In our study area, there are two natural features, the Fraser Canyon and Strait of Georgia, which divide the spawning habitat into three geographically discontinuous regions. We anticipated that gene flow between these regions might be restricted, leading to genetically distinct populations.

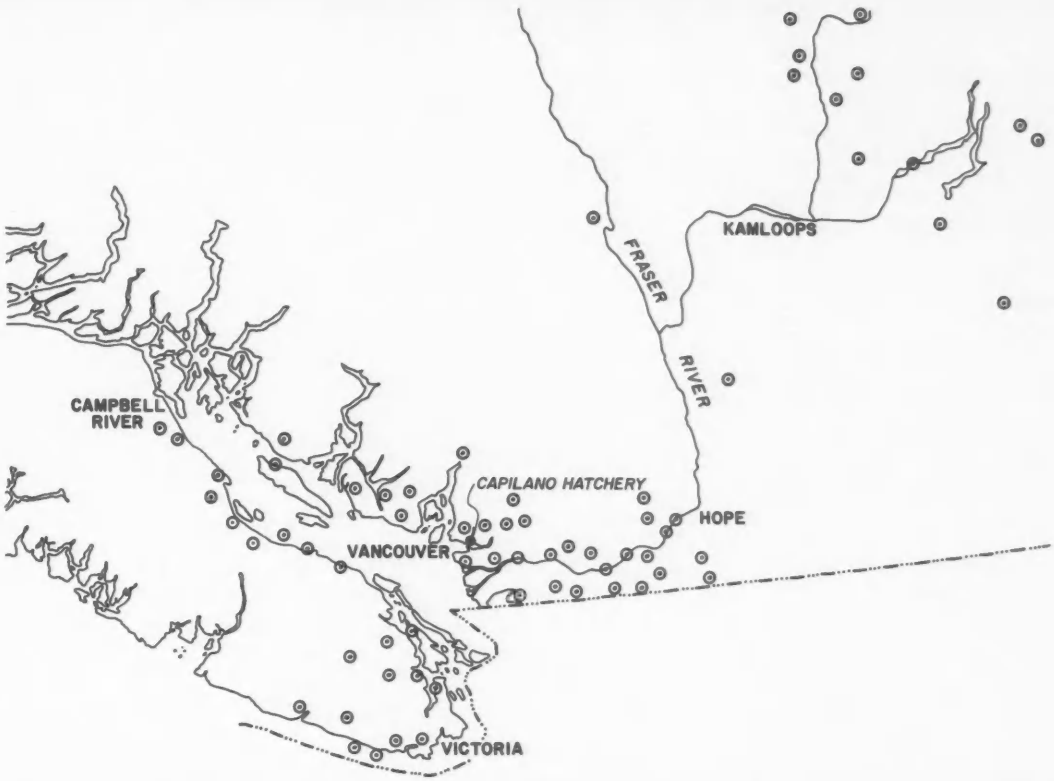

FIG. 1. Locations sampled for wild coho salmon fry in southern British Columbia. Where two or more locations were within 5 km of each other, only the central location is shown.

In this paper, we report the results of an electrophoretic survey of 26 enzyme loci in 95 populations of coho salmon sampled in the Fraser River watershed, the adjacent coastal mainland, and Vancouver Island. The main purpose of the study was to determine if various regional populations of coho salmon in southern British Columbia are genetically different. A second objective was to determine how much gene flow there is between populations within regions. Another objective was to provide baseline data on the genetic composition of coho salmon to allow future assessments of the effects of intensive fisheries management practices and large-scale salmon enhancement projects.

Materials and Methods

Fry from 94 sites in British Columbia (Fig. 1) were collected by electroshocking. For purposes of discussion, we will refer to three regions: "Vancouver Island," "Lower Mainland Coast," which includes all mainland sites tributary to the Strait of Georgia and the Fraser River west of the town of Hope, and the "Upper Fraser River" in central British Columbia, including all sites north of the town of Hope. Sites in the Upper Fraser Region are separated from the Lower Coastal sites by the precipitous Fraser Canyon, north of Hope, which has few significant coho spawning streams. In addition to the wild fish, we obtained 123 smolts from the Capilano Hatchery, just north of Vancouver (Fig. 1). The hatchery is the oldest coho-rearing facility in British Columbia and produces a substantial fraction of the coho salmon caught in the Strait of Georgia. The hatchery fish are treated separately because their progenitors included fish that were not native to the region.

Horizontal starch gel electrophoresis was used to assay enzyme genotypes. The methods used were adapted primarily from May et al. 1979. Twenty-six loci could be scored consistently (Table 1).

To identify and eliminate chinook salmon fry that were inadvertently included in some samples, we used *Pgi-3*, *Pgm-1*, and *Sod* which have different mobility patterns in coho and chinook (*O. tshawytscha*) salmon.

We obtained muscle and eye enzyme data for all fish. However, there was insufficient liver tissue in very small fish to enable us to get complete data for all liver enzymes in some populations. Therefore, in a few cases, we sampled additional fish, from a geographically adjacent population, for the same liver enzymes. In this way, liver enzyme data were collected fairly evenly over the geographic range of sampling sites. For most enzymes, we obtained data for 60 or more wild populations. The Capilano Hatchery fish were assayed for all 26 loci.

Migration Rate Estimation Methods

Estimators of migration rates were based on the "island"

model of population structure. In this model, a species has R populations, each consisting of N_e spawning adults of which a proportion m are immigrants from other populations. Immigration probabilities were assumed to be the same for all populations. Two estimators of the average number, $M = N_e m$, of migrants in each spawning population were used: the first one is based on the theoretical allele frequency variance among populations after correction for the sampling variance; the second one is a maximum likelihood (ML) estimator based on the theoretical distribution of allele frequencies.

Variance estimate of $M = N_e m$

We assumed that each of R populations consists of N_e spawning adults, of which a proportion, m, are immigrants from other populations. The average number of fish that are immigrants into each population is then $M = N_e m$ spawning adults every generation. If a sample of N fish is collected from each population, the variance in gene frequency, x, among populations is approximately

$$(1)\quad V(x) = \bar{x}(1 - \bar{x})[1/(1 + 4N_e m) + 1/2N].$$

The first term in (1) is the variance in gene frequency among populations (Crow and Kimura 1970) and the second term is the sampling variance. Equation (1) was rearranged to give an estimator of the number of immigrants in each spawning population, i.e.

$$(2)\quad \hat{M} = \frac{1 + 2N[1 - V(x)/\bar{x}(1 - \bar{x})]}{4[2NV(x)/\bar{x}(1 - \bar{x}) - 1]}.$$

For calculation purposes, all samples were assumed to consist of $N = 40$ fish. Only populations for which N was greater then 10 were used to compute $\hat{M}$.

Maximum likelihood estimate of $M = N_e m$

When a steady-state equilibrium between migration and random drift has been attained, the distribution of the allele frequency, x, is given by eq. 9.2.5 of Crow and Kimura (1970) for $0 < x < 1$. The probability, $f(0)$, that a variant allele is absent ($x = 0$) in a population of size N is given by eq. 9.2.16 of Crow and Kimura and is

$$(3)\quad f(0) = I_x(4M\bar{x}, 4M(1 - \bar{x}))\big|_{x = 1/2N}$$

where $I_x(.,.)$ is the incomplete beta function.

The logarithm of the likelihood that a set of populations will have allele frequencies of $x_1, \ldots, x_R$, for any $M = N_e m$, is

$$(4)\quad \ln L(M) = r\ln C(M) + (4M\bar{x} - 1)\sum_{i=1}^{r} x_i + (4M[1 - \bar{x}] - 1)\sum_{i=1}^{r}(1 - x_i) - r\ln 2N + (R - r)\ln f(0)$$

where $\bar{x}$ is the mean frequency of an allele, r is the number of populations which are polymorphic for the allele, and $R - r$ is the number of populations in which the allele is absent. In (4), $C(M)^{-1} = B(4M\bar{x}, 4M[1 - \bar{x}])$ where $B(.,.)$ is the beta function. Algorithms given by Best et al. (1986) were used to calculate values of the beta function and the incomplete beta function as well as to find the value of M which maximizes ln $L(M)$.

The standard error of $\hat{M}$ was determined by using the formula

$$(5)\quad S_{\hat{M}} = \left[-\frac{d^2 \ln L(\hat{M})}{dM^2}\right]^{-1/2}$$

where the value of the second derivative was calculated numerically as $[\ln L(\hat{M} + \Delta) - 2\ln L(\hat{M}) + \ln L(\hat{M} - \Delta)]/\Delta^2$ with $\Delta = 0.001$.

Results

Low levels of allelic variation occurred (Table 1). The locations where variant alleles occurred are mapped in Fig. 2. Of the 26 loci surveyed extensively, 16 were polymorphic in 1–11 populations. No loci were polymorphic in more than one fifth of all populations surveyed for the enzyme.

The mean heterozygosity, averaged over 26 loci (Table 1), is 0.25 ± 0.06% with a standard deviation among loci of 0.33%. The heterozygosity is extraordinarily low compared with most other fish species, but is consistent with the relatively low heterozygosity previously reported (Allendorf and Utter 1979) for Pacific salmon in general and for coho salmon in particular. The mean heterozygosity for 51 fish species, in the literature reviewed by Nevo (1978), was 5.13% compared with a range of 1.5% (for coho) to 4.5% (for chum) among the five Pacific salmon (Allendorf and Utter 1979). The transferrin locus, which is known to be highly polymorphic (Utter et al 1973a, 1973b; Suzumoto et al. 1977; Kirpichnikov 1981; Olin 1984), contributed to Allendorf and Utter's estimate of mean heterozygosity in coho salmon. We could not study this serum protein in our small fry. If we assume that the mean heterozygosity for the transferrin locus is the same (37%) in British Columbia as in Washington, then our estimate of overall mean heterozygosity increases to 1.62%, a value very close to that reported by Allendorf and Utter (1979). Olin (1984) estimated a mean heterozygosity of 4.3% for wild Oregon coho salmon, but he included the highly variable esterases as well as transferrin in his study.

Relative Levels of Genetic Variation in Coho Stocks

For enzymes assayed in our study, the percentage of each polymorphic allele is given in Table 2 for the Lower Coastal Mainland, Vancouver Island, the Upper Fraser River, Capilano Hatchery, and Olin's (1984) wild Oregon coho salmon. The fish of the Lower Coastal Mainland had a mean variant allele frequency of 0.151 ± 0.049% and a consequent heterozygosity for polymorphic loci of 0.302 ± 0.090%, which is greater than that for the other two regions of southern British Columbia (Table 2).

The following procedure (Sokal and Rohlf 1981) was used to test the hypothesis that the mean heterozygosity is the same in the Lower Coastal Mainland and each other stock. First, the frequencies (Table 2) of variant alleles were subjected to an arcsin square root transformation. Next, the transformed frequencies were "paired" with respect to loci and to alleles within loci to remove variation due to differences in mutation rate and selective effects. Finally, a "paired" t statistic for equality of mean frequency was calculated.

The probability of getting a higher t statistic than 2.97 on the hypothesis that Oregon and Lower Coastal Mainland fish have equal true mean heterozygosities is 0.008 (Table 2). Therefore, it is improbable that the 4.48-fold difference in variant allele frequency is due to chance.

The most plausible explanation for the extremely low level of genetic variation observed in the Lower Coastal Mainland is that a severe population bottleneck occurred in the last ice age. During the maximum glaciation of the Late Pleistocene, about

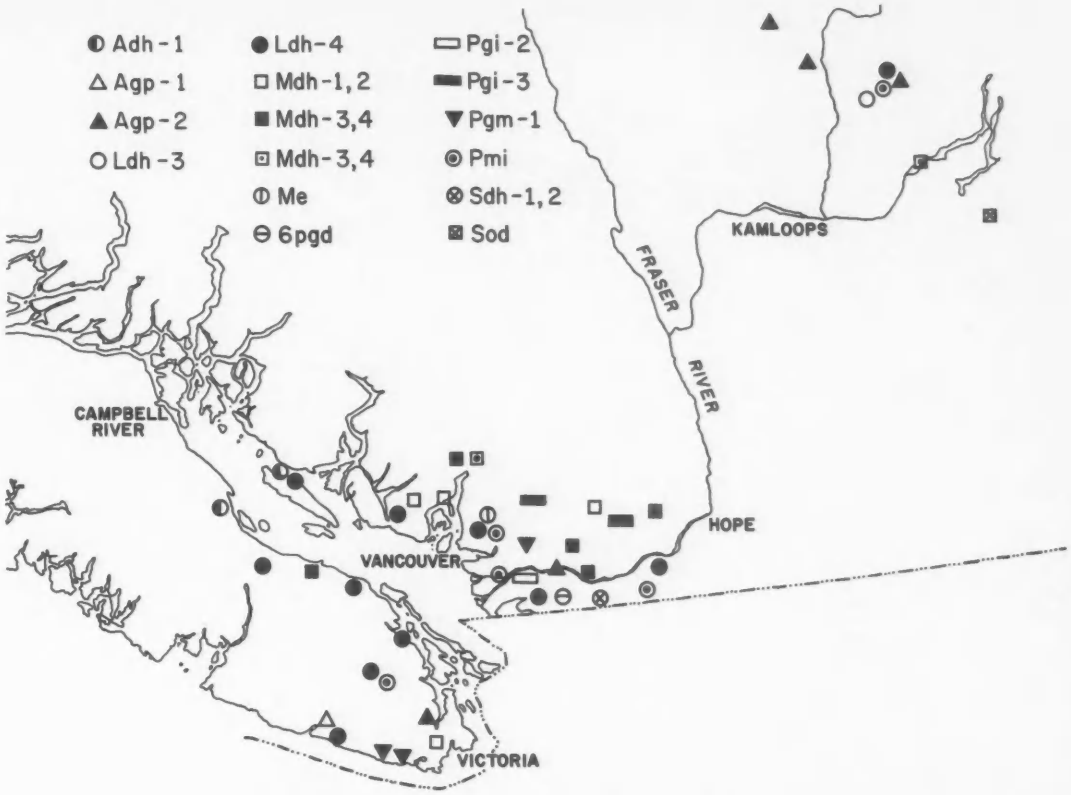

FIG. 2. Locations at which rare variant alleles of enzyme loci were found.

15 000 yr ago, ice covered the entire Fraser drainage and most of Vancouver Island. When the ice retreated, fish species recolonized the area from glacial refugia in the lower Columbia River system and coastal southern Washington (McPhail and Lindsey 1986). If the coho salmon of southern British Columbia descended from a sufficiently small founding population of strays from one of the glacial refuges, this would account for the fact that they have only 22% of the heterozygosity of fish from Oregon, which was not glaciated. The fact that variant allele frequencies among loci in the two regions have a negative, but not statistically significant, correlation ($r = -0.05$ in Table 2) is further evidence that the main source of genetic variation in British Columbia is not gene exchange with southern stocks.

The heterozygosity of Upper Fraser River fish is only 46% as high as it is in Lower Coastal Mainland fish. The difference is statistically significant and is probably due to a founder effect together with continuing low levels of gene flow through the Fraser Canyon. Nevertheless, the correlation ($r = 0.444$) among loci for the frequency of allelic variants between the Upper Fraser River and the Lower Coastal Mainland regions is statistically significant. This suggests that some of the variation that does occur in the Upper Fraser River is typical of, and presumably derived from, the Lower Coastal Mainland.

Extent of Genetic Differentiation among Geographic Regions

Populations with the same rare alleles, for one or more loci, occur in neighboring regions, such as Vancouver Island and the Lower Coastal Mainland, as well as the Lower Coastal Mainland and the Upper Fraser River watershed (Fig. 2). Clearly, there are no absolute barriers to dispersal and to gene flow in the area we sampled.

"Total contingency chi-square" values from tests for equality of gene frequencies between the Lower Mainland Coast, and each of the other stocks are presented (Table 2). For each variant allele with a frequency of nine or more in the two stocks to be compared, a 2 × 2 stock by allele contingency table was constructed and a contingency chi-square value (corrected for continuity) with 1 df was calculated (cf. Sokal and Rohlf 1981). With the exception of the Capilano Hatchery stock, all stock by allele classes had expected frequencies of more than 2.85. To obtain a simultaneous test of the equality of allele frequencies at all loci, the additive property of chi-squares was invoked to compute the total contingency chi-square.

Relative to fish from Vancouver Island, fish from the Lower Coastal Mainland have a greater frequency of the *Pgi-3*(90) allele, a lower frequency of the *Ldh-4*(120) allele, and moderate differences in frequencies of *Agp-2*, *Mdh-3,4*, and *Pmi* alleles (Table 2). On the hypothesis that allele frequencies in the two regions are equal for the five genes with nine or more variant alleles, the probability that the total contingency chi-square is greater than that observed (19.4 with 5 df) by chance alone is 0.001.

A test of the hypothesis that Upper Fraser River and Lower

TABLE 1. Enzyme locus, variant allele, location, region, and heterozygosity for all fish assayed for each enzyme.

Locus (EC number)	Allele	Location	Region	Heterozygosity	Number of fish	Number of sites
Adh-1 (1.1.1.1)	165	Overall		0.00394	508	16
		Cranby Ck.	VI	0.030 (1/33)		
		Trent R.	VI	0.024 (1/41)		
Agp-1 (1.1.1.8)		Overall		0.00044	2287	69
		Fairy L.	VI	0.025 (1/40)		
Agp-2	120	Overall		0.00831	2287	69
		Anderson Ck.	LCM	0.177 9/51)		
		Ayum Ck.	VI	0.027 (1/37)		
		Lemieux Ck.	UFR	0.077 (3/39)		
		Barriere R.	UFR	0.057 (2/35)		
		Clearwater R.	UFR	0.750 (3/4)		
		Capilano Hat.		0.008 (1/123)		
Idh-1 (1.1.1.42)	None				1970	65
Idh-2	None				1970	65
Idh-3	None				2226	62
Idh-4	130	Overall		0.00584	2226	62
		Capilano Hat.		0.106 (13/123)		
Ldh-1 (1.1.1.26)	None				2812	73
Ldh-2	None				2812	73
Ldh-3		Overall		0.00036	2812	73
		E. Barriere R.	UFR	0.025 (1/40)		
Ldh-4	120	Overall		0.00989	2731	70
		Little Campbell R.	LCM	0.024 (1/42)		
		Wade Ck.	LCM	0.025 (1/40)		
		Cranby Ck.	VI	0.026 (1/38)		
		Angus Ck.	LCM	0.050 (1/20)		
		Nile Ck.	VI	0.075 (3/40)		
		Bonsall Ck.	VI	0.024 (1/42)		
		Mesatchee L.	VI	0.026 (1/39)		
		Haslum Ck.	VI	0.025 (1/40)		
		Fairy L.	VI	0.122 (5/41)		
		Barriere R.	UFR	0.024 (1/41)		
		Capilano Hat.		0.089 (11/123)		
Ldh-5	None				2726	70
Mdh-1 (1.1.1.37)	None					
Mdh-2	140	Overall		0.00704	2842	73
		S. Alouette R.	LCM	0.016 (1/61)		
		Gosden Ck.	LCM	0.171 (7/41)		
		Angus Ck.	LCM	0.050 (1/20)		
		Ayum Ck.	VI	0.297 (11/37)		
Mdh-3	120	Overall		0.00844	2843	73
		Salmon R. (S)	LCM	0.072 (6/83)		
		Weaver Ck.	LCM	0.024 (1/41)		
		Kanaka Ck.	LCM	0.025 (1/40)		
		Nanoose Bay	VI	0.366 (15/41)		
		Stawamus R.	LCM	0.016 (1/61)		

TABLE 1. (*Concluded*)

Locus (EC number)	Allele	Location	Region	Heterozygosity	Number of fish	Number of sites
Mdh-4	175	Overall		0.00106	2843	73
		Adams R.	UFR	0.026 (1/39)		
		Stawamus R.	LCM	0.033 (2/61)		
Me-2 (1.1.1.40)	None				1946	58
6pgd (1.1.1.44)		Overall		0.00296	1013	28
		Mahood Ck.	LCM	0.079 (3/38)		
Pgi-1 (5.3.1.9)	None				2768	72
Pgi-2	125	Overall		0.00072	2768	72
		Murray Ck.	LCM	0.053 (2/38)		
Pgi-3	90	Overall		0.00361	2768	72
		S. Alouette R.	LCM	0.063 (4/64)		
		Coquitlam R.	LCM	0.353 (6/17)		
Pgm-1 (2.7.5.1)	135	Overall		0.00135	2223	66
		Scott Ck.	LCM	0.500 (1/2)		
		DeManiel Ck.	VI	0.025 (1/40)		
		Kirby Ck.	VI	0.024 (1/41)		
Pmi-1 (5.3.1.8)	110	Overall		0.00826	1090	29
		Mahood Ck.	LCM	0.071 (2/28)		
		Marshall Ck.	LCM	0.158 (3/19)		
		Mesatchee L.	VI	0.026 (1/38)		
		Barriere R.	UFR	0.024 (1/41)		
		Capilano R.	LCM	0.182 (2/11)		
Sdh-1 (1.1.1.14)	None					
Sdh-2	50	Overall		0.00165	606	21
		Salmon R. (S)	LCM	0.023 (1/44)		
Sod (1.15.1.1)	250	Overall		0.00471	850	28
		Salmon R. (N)	UFR	0.100 (3/30)		
		Gosden Ck.	LCM	0.024 (1/42)		

Coastal Mainland fish are genetically identical in the allele frequencies of the four genes with nine or more variant alleles used to compare the regions yielded a total chi-square of 10.8, which is significant at the 5% level (Table 2).

A most surprising discovery was that Capilano Hatchery coho smolts had a high frequency of the *Idh-4*(120) allele (Tables 1 and 2). This was the only population in southern British Columbia in which we found a mobility variant of the *Idh-4* gene. However, the *Idh-4*(120) allele is frequent in coho salmon collected in northern British Columbia (C. F. Wehrhahn and J. Jeyakumar, unpubl. data). There is also a much higher frequency of the *Ldh-4*(120) allele in Capilano Hatchery fish than in wild Lower Coastal Mainland fish. For the six alleles used to test for equality in allele frequency between Capilano Hatchery and wild Lower Coastal Mainland fish, the total contingency chi-square has a probability of less than 0.001 (Table 2).

Five alleles in Oregon fish have frequencies between 1.6 and 3% whereas none of the alleles in the Lower Coastal Mainland have frequencies greater than 1% (Table 2). Any of the alleles that are frequent in Oregon would serve to distinguish between fish in the two regions. The joint test of some or all alleles being more different in frequency than they are by chance alone yields a chi-square of 177.3, which is very highly significant (Table 2).These data make it doubtful that there is much interbreeding between fish in British Columbia and those in Oregon.

Test of the Neutral Steady-State Model

One test of the hypothesis that electrophoretic variation is selectively neutral and that sufficient time has elapsed for it to have reached a steady-state equilibrium is based on the ratio of the variance in heterozygosity among loci to the mean heterozygosity. For low mean heterozygosities the predicted steady-state *variance* in heterozygosity among loci is nearly *one third* of the *mean* heterozygosity if the mutation rates at all loci are the same (cf. Nei 1975; Wehrhahn 1975). The ratio of the variance to the mean heterozygosity ($\mathrm{Var}[H]/\bar{H}$) for the Lower Coastal Mainland is 1/152 (Table 2), which is less than 1/50 of the predicted ratio. The Oregon fish have less than 1/50 of the predicted variance. This suggests that even the Oregon cohos experienced a period in which their effective number was much lower than it is now. According to population genetic

TABLE 2. Percentages of variant coho alleles for the Lower Coastal Mainland (LCM), Vancouver Island, the Upper Fraser River, Capilano Hatchery, and Oregon wild fish (Olin 1984). Statistics include the mean percentage of each allele, the correlation [r (with LCM)] in allele frequencies of loci between the LCM and each other stock, the ratio [mean/mean (LCM)] of the mean percentage of variant alleles to that in the Lower Coastal LCM, and a paired t-test for equality of mean variant allele frequencies between the LCM and each other stock. The paired t-test and r (with LCM) were calculated on arcsin square root transformed allele frequencies. *Significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level.

Locus (allele)	LCM	Vancouver Island	Upper Fraser River	Capilano Hatchery	Oregon (wild)
Mean gene number	1729	1309	804	246	1286
Adh-1(165)	0	0.33	—	0	0
Agp-1	0	0.07	0	0	—
Agp-2	0.44	0.07	0.93	0	—
Idh-1(120)	0	0	0	0	0.31
Idh-2(80)	0	0	0	0	0.38
Idh-3(120)	0	0	0	0	1.94
Idh-4(130)	0	0	0	5.28	0.30
Ldh-1(150)	0	0	0	0	0.44
Ldh-2(50)	0	0	0	0	0.08
Ldh-3	0	0	0.11	0	0
Ldh-4(120)	0.14	0.76	0.11	4.47	0.08
Mdh-2(120)	0.40	0.63	0	0	0.31
Mdh-3(120)	0.40	0.86	0	0	0.15
Mdh-4(175)	0.09	0	0.10	0	0
6pgd	0.43	0	0	0	2.87
Pgi-2(140)	0	0	0	0	1.63
Pgi-2(125)	0.08	0	0	0	1.62
Pgi-3(90)	0.42	0	0	0	0
Pgm-1(135)	0.06	0.14	0	0	2.47
Pgm-1(160)	0	0	0	0	0.79
Pmi(110)	0.85	0.14	0.29	0	0.24
Sdh-1(150)	0	0	0	0	0.92
Sdh-2(50)	0.18	0	0	0	0.38
Sod(250)	0.14	0	0.06	0	0
Statistic					
Mean	0.151	0.125	0.070	0.406	0.678
±SE	±0.045	±0.052	±0.042	±0.282	±0.184
SD	0.222	0.255	0.199	1.382	0.862
r (with LCM)	1	0.450*	0.444*	−0.078	−0.050
Mean/mean(LCM)	1	0.83	0.46*	2.69	4.48**
Paired t-test		−1.070	−2.746*	−0.480	2.973**
df		13	12	12	19
Probability		0.305	0.017	0.644	0.008
Total contingency with LCM alleles					
Chi-square		19.4**	10.8*	160.8***	177.3***
df		5	4	6	11
Probability		0.002	0.030	0.000	0.000
Heterozygosity					
Var[H]/$\bar{H}$	1/152	1/96	1/87	1/11	1/46

theory (e.g. Wehrhahn 1975), the time that this occurred must have been much less than N generations ago, where N is the effective size of the species in the last few thousand years. Possibly the glacial refuges occupied by coho salmon were extremely small.

Gene Flow among Spawning Populations

In the absence of migration, a single gene at a locus in a population eventually becomes the ancestor of all $2N$ genes in the population. In each population, one allele will become monomorphic. However, different alleles can become monomorphic in different populations.

When migration is very high, a species will mate randomly. If this species is split into arbitrarily delimited populations, the allele frequency should be the same, except for sampling variation, in all populations.

TABLE 3. Estimates of the average number ($M = Nm$) of coho salmon immigrating into each local spawning population of southern British Columbia in each generation.

Locus	Polymorphic populations	$F(st)$	Number of immigrants: "Variance" estimate	Number of immigrants: Maximum likelihood
Agp-2	5	0.058	5.25	6.39 ± 3.83
Ldh-4	10	0.030	14.09	15.91 ± 5.99
Mdh-2	4	0.111	2.29	1.90 ± 1.23
Mdh-3	5	0.138	1.74	2.99 ± 1.77
Pmi-1	5	0.064	4.58	4.09 ± 2.49
Mean ± SE		0.080 ± 0.019	5.59 ± 2.23	6.25 ± 2.53
15 loci	43	0.086	3.16	5.80 ± 1.18

TABLE 4. Estimates of the average number ($M = Nm$) of coho salmon immigrating into each of the three major geographic regions of southern British Columbia in each generation.

Locus	Polymorphic populations	$F(st)$	Migration rate estimate: "Variance" estimate	Migration rate estimate: Maximum likelihood
Agp-2	3	0.0039	70.9	66.0 ± 48.4
Ldh-4	3	0.0040	68.8	106.5 ± 79.2
Mdh-2	2	0.0030	94.2	89.0 ± 65.3
Mdh-3	2	0.0045	60.5	62.0 ± 45.0
Pgi-3	1	0.0043	63.7	41.5 ± 55.8
Pgm-1	2	0.0008	591.5	457.4 ± 427.8
Pmi-1	3	0.0032	86.6	119.5 ± 90.8
Mean ± SE		0.0034 ± 0.0005	148.0 ± 74.1	144.9 ± 54.8

A "variance" estimate of the average number, $M = Nm$, of immigrants in spawning populations of coho salmon can be derived from the theoretical variance in gene frequency for populations of N individuals in which a proportion m are migrants from other populations (cf. Allendorph and Phelps 1981). Because our alleles were rare, our estimates (Table 3, column 4) have been corrected for sampling variation. The mean "variance" estimate for loci polymorphic in four or more populations is $M = 5.6 \pm 2.2$ immigrants per population (Table 3). The corresponding maximum likelihood estimate is $M = 6.3 \pm 2.5$ immigrants per population (Table 3). This is close to the "variance" estimate, but it should be more accurate, especially when populations with variant alleles are nearly randomly distributed geographically.

The estimates for different loci range from 1.90 immigrants for *Mdh-1,2* to 15.91 immigrants for *Ldh-4* (Table 3). The difference of 14.01 is 2.23 times the standard error (6.28) of the difference between the two loci. According to Tukey's HSD a posteriori test (Sokal and Rohlf 1981), for all possible comparisons among five normally distributed means, the critical difference is $3.09/\sqrt{2} = 2.19$ times the standard error of the difference. Hence, the migration rate difference between the loci is significant at the 5% level and we tentatively conclude that the rate of gene flow is higher for *Ldh-4* than for other loci. A possible explanation is that there is a higher migration rate between some kinds of habitats than others and that the *Ldh-4*(120) allele happens to occur mainly in habitats between which there is high migration.

Final estimates of the mean migration rate were obtained by using variant allele frequencies pooled over all loci. In particular, the maximum likelihood estimate of the number of immigrants in each spawning population is 5.8 ± 1.2 when information on all 15 of the loci that are polymorphic in wild fish is used (Table 3).

Gene Flow among Regions

It is convenient to pool all of the "subpopulations" within each of the natural geographic regions of southern British Columbia and to regard the assemblage as one "population." When this is done, estimates of the number of migrants spawning in each region can be calculated as before (Table 4). Estimates are given for the seven loci that had variant alleles at four or more sites. The maximum likelihood estimates range from 40 migrants for *Pgi-3* to 460 migrants for *Pgm-1*, but the difference is not statistically significant according to Tukey's a posteriori test. The mean "variance" estimate for the seven loci is 148 ± 74 migrants per generation and the corresponding mean maximum likelihood estimate is 145 ± 55.

Although the estimates are for the simple "island" model of population structure, more complicated hierarchical and one- or two-dimensional models of population structure are probably more realistic (cf. Maruyama 1977).

Historically, "effective population sizes" of breeding salmon in the Lower Coastal Mainland and the Vancouver Island regions have been on the order of many hundreds of thousands. However, studies on recruitment to Upper Fraser River spawning areas between 1950 and 1980 suggest that the effec-

tive population size of the region may be as low as 10 000 (cf. Fraser et al. 1982). This means that the proportion of successful immigrants into each of the major geographically delimited regions, from neighboring regions, is 1/100 to less than 1/1000 of the breeding population in a region. These are sufficiently low migration rates that even mild selection ($s > 0.01$), for traits adapted to a specific region, should counter gene flow and allow geographically specialized races to arise.

This kind of geographic specialization has apparently taken place. Taylor and McPhail (1985a, 1985b) have shown that Upper Fraser River coho salmon have slower burst swimming speeds but greater stamina than coastal fish. These differences are inherited and appear to be related to inherited differences in body morphology. Furthermore, C. Lindsey (Zoology Department, University of British Columbia, pers. comm.) has found that the Upper Fraser River coho salmon are very different in meristic traits from their relatives in the Lower Coastal Mainland.

Conclusions

The coho salmon of southern British Columbia are much less polymorphic than Oregon fish (Table 2). The data on enzyme loci are consistent with the hypothesis that a major bottleneck in population size occurred during the colonization of British Columbia after the last ice age. The fish of the Upper Fraser River have only 46% of the heterozygosity of Lower Coastal Mainland fish (Table 2). This is undoubtedly due to additional historical population bottlenecks, as well as to an estimated low migration rate into the region of 145 ± 55 spawning adults per generation.

The coho salmon of the Lower Coastal Mainland are genetically distinguishable from those on Vancouver Island. They are also distinguishable from fish in Oregon. The coho salmon in the Capilano Hatchery, which is the oldest major salmon hatchery in the Lower Coastal Mainland, differ from wild fish in the region (Table 2).

An implication of these findings is that it should be possible to determine the relative contribution of different stocks to sufficiently large oceanic coho samples.

The maximum likelihood estimate of the average number of "strays" or immigrants that are present in each local spawning population is $Nm = 5.8 \pm 1.2$ (Table 3), with a 95% confidence interval of 3.5−8.1. All estimates were based on the "island" model of population structure. This model is not really realistic because populations with a particular rare allele tend to be near other populations with the allele (Fig. 2), suggesting that gene exchange is mainly between nearby populations. However, departures from the "island" model need not lead to systematic errors in migration rate estimates. Slatkin (1985) carried out simulation studies of rare alleles as indicators of gene flow. He found that the number, Nm, of immigrants per population is related to the frequency of alleles that are "private" to individual populations. The relationship was insensitive to factors other than the migration rate and the number of individuals sampled from each population. Furthermore, the relationship for the "island" model was indistinguishable from that for the two-dimensional stepping-stone and the hierarchical population structure models which are more realistic for coho salmon.

For selectively neutral alleles, the variation among populations is proportional to $F(st) = 1/(1 + 4Nm)$ (Crow and Kimura 1970). For values of Nm less than 0.1, $F(st)$ is close to 1 and there is considerable variation among populations, but little heterozygosity within populations. In contrast, for $Nm = 6$, as in coho salmon, 1/25 of the genetic variance is attributable to population subdivision and the rest occurs within populations as heterozygosity. The actual estimate of $F(st)$ is 0.08 ± 0.02 (Table 3), which is twice the theoretical value of 0.04 based on the rate of gene flow.

A more important aspect of gene flow is that its magnitude influences the degree to which ecotypic differentiation can take place. Consider a semidominant allele whose heterozygote has an advantage of s in a local population. If $4Ns$ is greater than 1, random drift will not overwhelm natural selection (Crow and Kimura 1970). However, in the presence of five or more immigrants per generation, as in coho salmon, migration will prevent a locally adapted allele from becoming frequent unless $4Ns > 4Nm = 20$. If the effective size of a spawning population is greater than 1000, a very small selective advantage, in heterozygotes, of $s > 20/(4 \times 1000) = 0.005$ would counter migration and eventually make an allele common. This means that in the presence of five immigrants per generation, a population of 1000 or more spawners can be considered to be "large" enough so that constraints on ecotypic differentiation are negligible. A consequence is that, if the habitats occupied by such populations are stable for a long time, specific adaptations to local conditions should be present. The implication for fisheries managers is that spawning populations of 1000 or more coho salmon are "large" from an evolutionary point of view and should be protected from extinction even if they exhibit no obviously unique ecotypic characteristics.

Fraser et al. (1982) compiled data on coho escapement to much of the Upper Fraser River and its tributaries from 1950 to 1980. A few breeding populations are fairly large. An example is the Eagle River population with an escapement of 750−2500 in most years. Allowing for variation in population size and nonrandom production of offspring, this corresponds to an effective population size, N, of more than 1000 which is "large." However, the majority of streams listed by Fraser et al. have smaller breeding populations of 200 or fewer fish. Many of these populations could have effective sizes of 50 or less. If there are five immigrants per generation in a breeding population of 50, then $m = 5/50 = 0.1$, and for an allele to be retained in a population, the selective advantage of its heterozygotes would have to be greater than 10%. Such populations can be regarded as "small" because immigration should usually overwhelm adaptation to local conditions. Small populations of coho salmon occur predominantly in small streams with a limited habitat for spawning and rearing fish. Genetically these fish are probably broadly adapted to a range of habitats, but have few characteristics which are unique to individual streams.

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Dynamics of Drift and Microdistribution of Two Mayfly Populations: A Predictive Model

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A model is derived to predict changes in larval microdistribution of *Ephemerella inermis* Eaton and *Baetis tricaudatus* Dodds (Ephemeroptera) that result from drift. Transport by drift was assumed to be a Markov process. Estimates of departure from the substrate, downstream movement, and lateral transport were combined to produce transition matrices from which changes in microdistribution, magnitude of drift, and mean daily downstream displacement of live and dead animals were determined. Predictions from the model were compared with observations made in a river similar in composition to the area modelled. There were marked differences between expected drift of living versus dead animals; estimates for drift of living animals corresponded most closely to values observed in the field. Predicted drift either corresponded to or was less than observed levels. Observed changes in benthic microdistribution were minimal, but the model predicted large-scale relocation of animals toward river margins. Thus, magnitude and spatial pattern of drift could be adequately modelled but benthic distribution could not. Drift is an important redistribution mechanism but animals must have a complementary ability to actively travel towards the river center to maintain constant benthic distribution patterns. Inhibition of movement during floods should produce increased abundance at margins. During stable flow conditions, drift and benthic activity probably act together to produce and maintain microdistributional patterns among vagile organisms. In contrast, drift of invertebrates possessing poor locomotory ability probably reflects larger-scale, permanent displacement events that are a normal aspect of development, or the outcome of aggressive interactions.

On a élaboré un modèle de prédiction des variations de la microdistribution larvaire de *Ephemerella inermis* Eaton et de *Baetis tricaudatus* Dodds (Ephemeroptera) qui résultent de la dérive. On a présumé que le transport par dérive est un processus de Markov. Des estimations de l'émigration du substrat, des déplacements vers l'aval et du transport latéral ont été combinées pour obtenir des matrices de transition qui ont servi à déterminer les variations de la microdistribution, l'importance de la dérive et les déplacements quotidiens moyens d'animaux vivants et morts vers l'aval. Les prédictions tirées du modèle ont été comparées aux observations réalisées dans une rivière semblable à la zone modélisée. Il existait des différences marquées entre la dérive attendue des animaux vivants par rapport aux animaux morts. Les estimations de la dérive des animaux vivants correspondaient plus étroitement aux valeurs observées sur le terrain. La dérive extrapolée était égale ou inférieure aux niveaux observés. Les variations notées dans la microdistribution benthique étaient minimes, mais le modèle prédisait le déplacement à grande échelle des animaux vers les rives. Ainsi, l'amplitude et la répartition spatiale de la dérive peuvent être adéquatement modélisées mais non la répartition benthique. La dérive constitue un important mécanisme de redistribution mais les animaux doivent posséder une capacité complémentaire de déplacement actif vers la partie centrale d'un cours d'eau pour maintenir une répartition benthique constante. La cessation des déplacements au moment de crues devrait entraîner une plus forte abondance au niveau des rives. En présence d'un débit stable, la dérive et l'activité benthique concourent probablement à produire et à maintenir des régimes de microrépartition chez les organismes vagiles. En revanche, la dérive d'invertébrés à faible capacité locomotrice traduit probablement des déplacements permanents à grande échelle qui représentent un aspect normal du développement ou sont le résultat d'interactions agressives.

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Drift is an important aspect of the population dynamics of many lotic invertebrates. It serves to remove individuals from unfavorable microhabitats (Corkum et al. 1977; Walton et al. 1977; Peckarsky 1980; Kohler 1985) and provides animals that may colonize unexploited areas downstream (Ulfstrand et al. 1974; Townsend and Hildrew 1976; Williams and Hynes 1976; Ciborowski and Clifford 1984). Twenty-five years of intensive research on factors inducing departure of animals from the substrate has produced a wealth of data. Virtually every parameter that can be measured within a stream system has been correlated with the drift of one or more species (Statzner et al. 1984). Yet, the variety of responses seen among both taxa and stream systems has made it difficult to integrate this information to provide a comprehensive interpretation of the phenomenon.

An implicit assumption made regarding drift has been that the force of the current in lotic systems restricts benthic movement of invertebrates to the extent that drift produces a permanent downstream displacement of populations. Accordingly, Müller (1954, 1973, 1974) hypothesized the existence of a colonization cycle. Newly hatched, rapidly growing individuals drift because they are restricted in resources of food

and space. Adults fly upstream to oviposit and complete the cycle. Waters (1965, 1972) observed that drift is not always predominant among early developmental stages. Neither do all species show a preponderance of upstream flight by adults. Consequently, he suggested that drift is a density-dependent phenomenon that removes animals in excess of carrying capacity; drifting animals are lost to the system.

However, neither of these hypotheses can consistently explain why many stream systems apparently support continuously high levels of density-independent drift. A large part of the problem in deriving a comprehensive explanation of the significance of drift has been a failure to adequately integrate the proximal causes of drift episodes with the outcome of the event. Kovalak (1979) proposed that benthic distribution of rheophilic populations was largely maintained by a balance between immigration and emigration through drift mediated by behavioral responses to current velocity. Several investigators have observed that drift can result in seasonal or short-term, small-scale displacement of significant proportions of populations (Ulfstrand 1968; Lehmkuhl and Anderson 1972; Hall et al. 1980; Ciborowski and Clifford 1983).

In this study, I examine the role of daily drift in maintaining the distribution of two mayfly populations among microhabitats in a wide, shallow, relatively homogeneous river. A model is developed to predict fluctuations in transverse distribution of larvae with time, given data on their departure and settling capabilities relative to current velocity. In order to distinguish purely physical influences of water flow from organisms' behavioral responses, all experimental manipulations were conducted with dead individuals concurrently with live animals. Whereas predicted distributions of dead animals would reflect purely mechanical aspects of drift, differences between such distributions and those observed for living animals must be ascribed to individuals' behavioral activity.

A Model Relating Drift to Benthic Microdistribution

Lotic invertebrates may relocate by crawling over or through the substrate, or by drifting. As a working hypothesis, I initially postulate that only the latter mechanism might produce a net change in distribution of animals, i.e. other forms of movement are nondirectional. Drift consists of three events: departure from the substrate, transport in the water column (drift proper), and return to the substrate. Because the actual moment of return to the substrate cannot easily be distinguished from the period of transport, I will refer to these components together as settling.

Current velocity exerts a major influence on benthic microdistribution, departure from the substrate, and settling, both through its direct actions on organisms and indirectly by modifying substrate characteristics or food abundance, to which animals also respond. Consequently, I assume that regions of comparable current velocity will contain equal benthic densities of organisms, each individual exhibiting species-specific departure and settling characteristics.

Entry into the water column may be passive, effected directly by erosion acting upon animals in vulnerable positions on the substrate (Elliott 1967). In such cases, departure tendencies should be greater from regions of high current velocity than from low. Alternatively, some rheophilic organisms may actively desert a microhabitat in which velocity becomes unfavorably reduced (Edington 1965; Minshall and Winger 1968). Other factors such as food abundance (Bohle 1978; Corkum and Clifford 1980; Walton 1980; Kohler 1985) and substrate texture (Corkum et al. 1977; Walton et al. 1977) also modify departure tendencies. These features are frequently correlated with or assume subsidiary importance to current velocity (e.g. Corkum et al. 1977; Horner and Welch 1981).

An animal suspended in the water column can undergo lateral transport as well as downstream movement during the settling event, which may result in a return to the substrate either shoreward or centerward from its point of departure. Degree of lateral displacement depends on time spent in the water column, turbulence, and behavioral actions of the animal during transport (Ciborowski 1983a). The former two factors are directly dependent on current velocity. By simultaneously considering departure from and subsequent settling among all microhabitats, it should be possible to anticipate the role of drift in maintaining microdistribution among habitats.

If a river is longitudinally homogeneous relative to the distance an animal is carried prior to settling, upstream–downstream microhabitat differences will be minimal, and changes in benthic distribution can be considered primarily in terms of organisms' lateral movement. Consider an ideal river, longitudinally partitioned into a discrete number of parallel homogeneous regions (i) of arbitrary width (see Appendix 1.1 for a complete list of symbols). Each has an associated uniform mean current velocity (v_i). Velocity is minimal at river margins and maximal at the center.

Let $B^0 = (b_1^0, b_2^0, b_3^0, \ldots b_i^0)$ be a vector with each b_i^0 the benthic density of animals in region i on a given day (0). Because each region is uniform along its length b_i^0 is constant throughout.

If conditions in the river are constant through time, a fixed proportion of animals will either depart from the substrate and enter the water column ($p(t)_{ii}$) or remain in the substrate ($q(t)_{ii}$) during time interval t at every point along a given region (i). These proportions can be assembled as diagonal matrices, $\boldsymbol{P}(t)$ and $\boldsymbol{Q}(t)$. (It is convenient to consider t as 1 d). Because velocity varies predictably across the river and is a major factor controlling likelihood of departure from the substrate, p_{ii} and q_{ii} can be estimated by considering the position of any particular region relative to a river margin.

Let k_{ij} be the probability that an animal that has entered the drift from region i returns to the substrate (settles) in the original or any other region (j). Because downstream and lateral transport are functions of current velocity, k_{ij} will be constant for each organism in a given homogeneous region. Elements k_{ij} can be assembled into a square probability matrix, $\boldsymbol{K}$. Matrices $\boldsymbol{P}(t)$, $\boldsymbol{Q}(t)$, and $\boldsymbol{K}$ can then be combined to form a matrix, $\boldsymbol{C}$, such that each element, c_{ij}, is the probability that any animal presently in the substrate in region i will be located in the substrate in region j on the next day:

$$(1) \quad \boldsymbol{C} = \boldsymbol{P}(t)\boldsymbol{K} + \boldsymbol{Q}(t).$$

That is, the probability of an animal being located in any region j after time t is the probability of departure from the substrate in any region i times the probability of transport from i to j plus the probability that the animal does not drift (i.e. remains in region i). (By including the values of $\boldsymbol{Q}(t)$, one is making animals that do not drift functionally equivalent to those that do drift but do not change regions.) I assume that any individual that does drift does so only once per day. Allowing that each c_{ij} remains constant through time, $\boldsymbol{C}$ is the transition matrix of a Markov chain, and B^t, the vector describ-

ing benthic density of animals among regions after t days, is

$$(2) \quad B^t = B^0C^t$$

where C^t is the tth power of C. This requires the further assumption that location of an individual on any one day depends only on its location the previous day, and not on its location at any time prior to this.

Provided that C has no absorbing states (diagonal elements = 1.0), then, given ample time, an equilibrium will be reached in which the benthic distribution of animals among regions will remain constant. This equilibrium depends only on the structure of C and is independent of B^0. If, however, one or more absorbing states does exist (i.e. animals that enter a region cannot leave it by drifting), each animal will eventually become restricted to one of these regions.

Prediction of benthic distribution from the model requires the following data: (1) current velocity (v_i) profile of the river under investigation; (2) benthic density (b_i) of animals in regions across the river; (3) likelihood of departure from the substrate in each region ($p(t)_{ii}$); (4) settling abilities and lateral dispersal of animals that depart from the substrate in each region (k_{ij}).

Methods and Analysis

Study Site and Animals

Data were collected from a study site on the south shore of the Pembina River near Entwistle, Alberta, Canada (53°37′N, 115°00′W). The site is wide and shallow (maximum depth 1.2 m) with a spatially uniform substrate of smooth cobbles embedded in coarse sand. It was selected for its evenness of current velocity and depth profiles, both of which increase linearly with increasing distance from the river margin (Ciborowski 1983a). The sample site is approximately 65 m wide during normal flow conditions. However, data on water flow, microdistribution, drift, and settling capabilities of the study animals were collected primarily from within 12 m of the southern riverbank.

Larvae of the mayflies *Baetis tricaudatus* Dodds and *Ephemerella inermis* Eaton were abundant during most of the year. Larvae of both species usually are most numerous in the benthos in regions of faster water, remote from the river margin, although large numbers are common in the drift across the river (Ciborowski and Clifford 1983). Both taxa exhibit strong nocturnal drift periodicities. Dead animals almost never appear in the benthos or as part of the natural drift. Only relatively large larvae could be collected in sufficient numbers for departure and settling experiments (see below). In May 1979, experimental animals had head widths >0.7 mm (both species used). Head widths in October 1979 were 0.4–0.6 mm (*B. tricaudatus* only used).

Model River

Instead of extrapolating data to provide estimates for the entire width of the river, I based my predictions on an hypothetical river with flow characteristics analogous to those of the sampled portions of the Pembina in May and October 1979 (Ciborowski 1983a). The model river was 26 m wide and partitioned into 1-m-wide regions. Depth and mean current velocity between 0 and 12 m from the south margin increased in a manner similar to that estimated for the Pembina River and at twice this rate between 0 and 6 m from the north bank (Fig. 1). The central 8 m were of uniform depth and velocity,

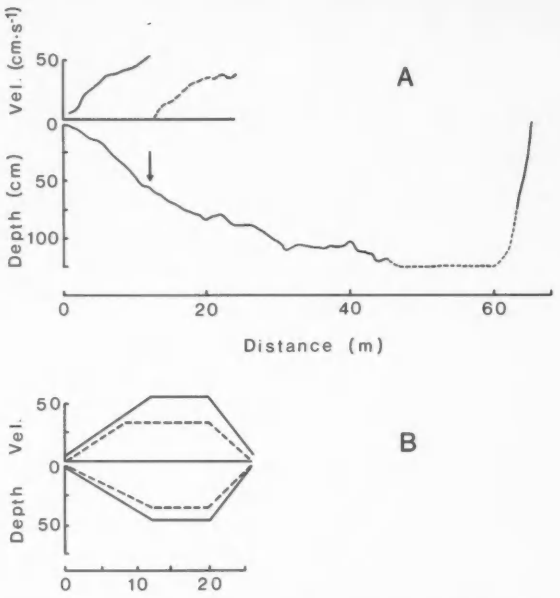

FIG. 1. (A) Depth (below) and current velocity (above) profiles of the Pembina River at the sample site. Left margin of graphs (distance of 0 m) corresponds to position of the south river margin in May. Arrow (below) and broken line (above) represent position of the river shoreline and corresponding current velocity profile in October. (B) Depth (below) and current velocity profile (above) of the model river in May (solid lines) and October (broken lines).

equivalent to values 12 m from the south margin of the Pembina River.

Benthic Distribution

Benthic microdistribution of *E. inermis* and *B. tricaudatus* larvae in the Pembina River was studied at 10- to 15-d intervals throughout 1979 (Ciborowski and Clifford 1983). Five replicate Hess samples (net pore size 0.380 mm) were taken 3 and 11 m from the south margin until mid-June and usually at 3, 7, and 11 m thereafter. Numbers of large animals in each sample within series of replicates on each date were adjusted to reduce sample variances (Sokal and Rohlf 1969) by regressing benthic densities against numbers of all other benthic animals taken in each sample (Ciborowski and Clifford 1983).

Benthic densities in the model river were allocated in relative quantities analogous to those of the Pembina River. I initially determined the relationship between adjusted benthic density of each Pembina River population and distance from the south river margin by linear regression (Table 1). The 12 regions extending from the south side of the model river were assigned densities interpolated directly from these regressions. Densities between 13 and 20 m from the south margin were the same as at the 12-m point. North margin densities were obtained by doubling the slope of the regression equations. "Dead" animals (those that would be affected only by mechanical forces in model runs) were given the same initial benthic distributions as their living counterparts.

Departure from the Substrate

Ciborowski (1983b) studied the influence of benthic density,

TABLE 1. Regression coefficients and coefficients of determination of functions relating benthic distribution (b_i) of animals in each region (i) within 12 m of the south margin of the model river to distance from the south margin (y_i). Equations take the form $b_i = \alpha_0 + \alpha_1 y$. Data were obtained from benthic samples collected in the Pembina River.

Species	Date	Intercept α_0	Slope α_1	R^2
E. inermis	15 May	−13.00	7.00	0.86
	28 May	3.25	8.25	0.80
B. tricaudatus	15 May	− 13.49	6.72	0.41
	28 May	− 23.53	8.50	0.63
	4 Oct.	− 16.62	20.01	0.63
	15 Oct.	−115.75	41.30	0.46

detritus concentration, and current velocity on departure of both living and dead individuals from the two populations in laboratory streams. Predatory stoneflies were present in all of these experiments to render observed drift levels more representative of field conditions. Current velocity significantly influenced the departure of the two taxa, but neither benthic density of conspecifics nor amount of detritus affected drift levels. I used regression equations from that study, which related current velocity to departure, to provide estimates of daily departure from regions in the model river. Expected proportions of each population departing from the substrate in each model river region were determined by substituting the appropriate mean current velocity (Fig. 1) into these equations. Where departure was best described as a polynomial function of current velocity, mean departure proportion was estimated by integration. Because the duration of the laboratory experiments was slightly less than 24 h (Ciborowski 1983b), departure proportions were increased to correspond with field photoperiods (16:8 and 12:12 L:D in May and October, respectively (Fig. 2)).

The model permits animals to drift only once during a 24-h period. Let $p(L)_{ii}$ and $p(D)_{ii}$ be the proportions of animals departing from region i during day (L) and night (D), respectively. The proportions that remain on the substrate are

$$q(L)_{ii} = 1 - p(L)_{ii}$$

$$q(D)_{ii} = 1 - p(D)_{ii}.$$

Assuming that the "drift day" begins at dusk, the proportions that depart or remain during 24 h are

$$(3) \quad p(24)_{ii} = P(D)_{ii} + q(D)_{ii}\, p(L)_{ii}$$

$$(4) \quad q(24)_{ii} = 1 - p(24)_{ii}.$$

Settling

Settling animals, whether living or dead, return to the substrate according to a negative exponential function (McLay 1970; Elliott 1971a; Larkin and McKone 1985). The proportion that settles (f_j) over unit length of any region i depends on the instantaneous settling rate r_i. If $m(x)_i$ is the proportion of animals suspended in the water column over unit length in region i, x metres downstream of the point of entry into the water column ($m(0)_i = p_{ii}$), then

$$(5) \quad m(x)_i = p_{ii}\, e^{-rx}.$$

Consequently, the proportion that settles over a unit of downstream distance is

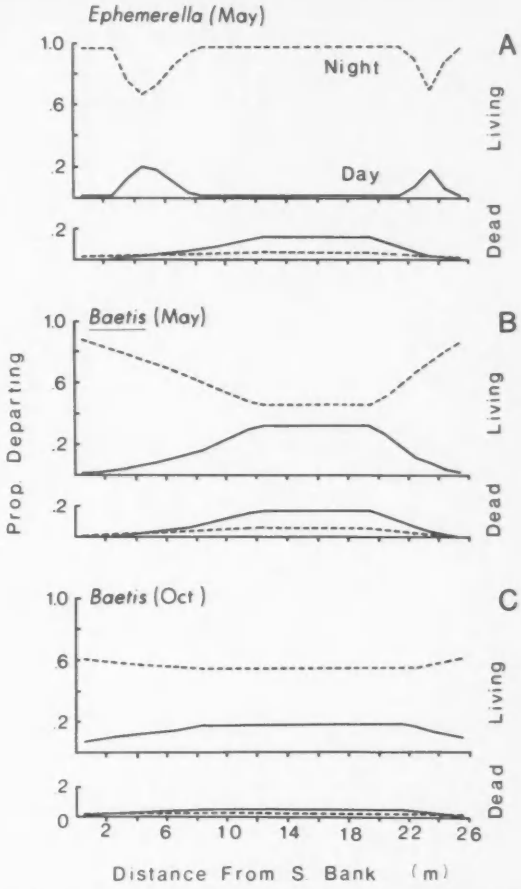

FIG. 2. Proportion of animals that depart from substrate in the model river during day (solid lines) and night (broken lines) based on laboratory studies relating departure to current velocity. (A) *Ephemerella inermis* (May). Nonlinearities in nocturnal drift density pattern reflect a polynomial response to velocity. Daytime nonlinearities are an artifact of the model restriction that animals drift only once per 24 h. (B) *Baetis tricaudatus* (May); (C) *B. tricaudatus* (October). Figures above represent departure of living individuals; figures below represent departure of dead animals.

$$(6) \quad f(x)_i = m(x - 1)_i - m(x - 1)_i\, e^{-r} = m(x - 1)_i\,(1 - e^{-r}).$$

Settling rate is itself a power function of current velocity (v) (Elliott 1971a; Ciborowski and Corkum 1980). However, because current velocity in the model river is a linear function of distance from shore (y) except at the river center (Fig. 1), settling rate can also be expressed as

$$(7) \quad r_i = \beta_1 y^{-\beta_2}$$

where β_1 and β_2 are regression constants specific to the animal population considered.

The lateral distribution of suspended animals about their point of introduction into the water column is normal, with mean given by the initial distance from shore (y_i) and the standard deviation as s_i (Ciborowski 1983a). The standard

TABLE 2. Regression coefficients and coefficients of determination of functions estimating instantaneous settling rate (r) and measures of lateral dispersal (s_i) of suspended animals originating from each region (i; $i \leq 12$) of the river. Equations take the form $r = \beta_1 y_i^{\beta_2}$ and $s_i = \beta_3 + \beta_4\sqrt{x} + \beta_5 y_i$ where y_i = distance of a region from the south river margin and x = downstream distance of an animal from its point of departure from the substrate. Data were obtained from field experiments conducted in the Pembina River.

	Settling			Lateral dispersal			
Regression coefficient	β_1	β_2	R^2	β_3	β_4	β_5	R^2
Living							
E. inermis (May)	1.837	−1.526	0.93	−0.767	0.570	0.117	0.56
B. tricaudatus (May)	1.231	−0.983	0.94	−0.526	0.589	0.059	0.79
B. tricaudatus (Oct.)	0.504	−0.642	0.98	−0.547	0.388	0.064	0.49
Dead							
E. inermis (May)	1.206	−1.620	0.88	−0.045	0.243	0.015	0.59
B. tricaudatus (May)	0.387	−0.798	0.72	0.144	0.096	0.015	0.56
B. tricaudatus (Oct.)	0.247	−0.805	0.62	−0.272	0.243	0.035	0.60

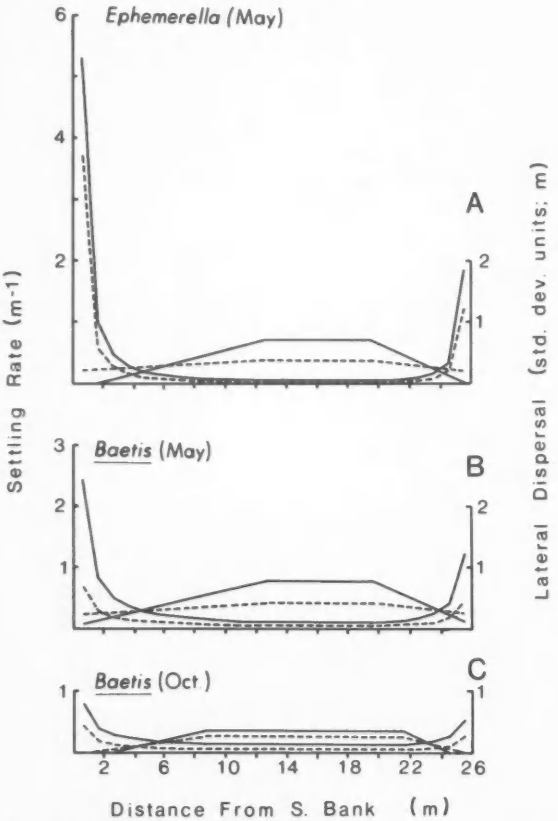

FIG. 3. Instantaneous settling rates, r (concave curves, left axes), and measures of lateral dispersal, s (convex curves, right axes), ascribed to living (solid lines) and dead (broken lines) larvae suspended in the model river. Values were interpolated from release–recapture experiments conducted in the Pembina River. (A) *Ephemerella inermis* (May); (B) *B. tricaudatus* (May); (C) *B. tricaudatus* (October).

deviation is dependent on both the square root of downstream distance travelled ($x^{0.5}$) and mean current velocity (v). The linear relationship between v and y permits s to be expressed as

$$(8) \quad s_i = \beta_3 + \beta_4 x^{0.5} + \beta_5 y_i$$

where β_3, β_4, and β_5 are population-specific regression constants (Ciborowski 1983a).

I reanalyzed data collected on settling rates and lateral dispersal of suspended live and dead *E. inermis* and *B. tricaudatus* larvae in the Pembina River in May and October 1979 (Ciborowski 1983a) to express r and s as functions of distance from shore (Table 2). I then estimated r and s in each season for each region of the 12 m from the south shore of the model river by substituting the distance from shore of the midpoint of each region into the regression equations. Estimates for regions 0–6 m from the north margin were determined by substituting twice the midpoint distance into regression equations. Values for the eight central regions were calculated by setting $y = 12$ (Fig. 3). Equations 7 and 8 were used to generate two matrices, $\boldsymbol{K}$ and $\boldsymbol{W}$ (Appendix 1.2). Matrix $\boldsymbol{K}$, elements of which represent probabilities of transport from region i to region j, was used in equation 1. Matrix $\boldsymbol{W}$, elements of which represent the sum of proportions of animals departing from region i that would be carried past a line stretching across region j, was used to predict expected numbers of animals that would be caught in drift nets placed across the model river (see below). Matrices $\boldsymbol{K}$ and $\boldsymbol{W}$ were estimated for both living and dead *E. inermis* and *B. tricaudatus* larvae.

Observed and Predicted Changes in Distribution

All settling and departure estimates were based on results of experiments conducted in May and October 1979. Benthic field samples that most closely corresponded with these periods were collected 15 and 28 May and 4 and 15 October. Consequently, predictions of relative changes in benthic density in the model river were made for 13- and 11-d intervals, respectively, using equation 2. Initial distributions (B^0) corresponded to distributions on 15 May and 4 October, respectively. Expected distributions (B^{13} and B^{11}) were then comparable with observed distributions on 28 May and 15 October, respectively.

Observed and Predicted Drift

Observed drift

Drift was monitored in the Pembina River 28–29 May and 7–8 October 1979 with twelve 1-m-wide contiguous nets (mesh pore size 0.211 mm) extending from the south margin (Ciborowski 1983a). Samples of up to 30-min duration (timed

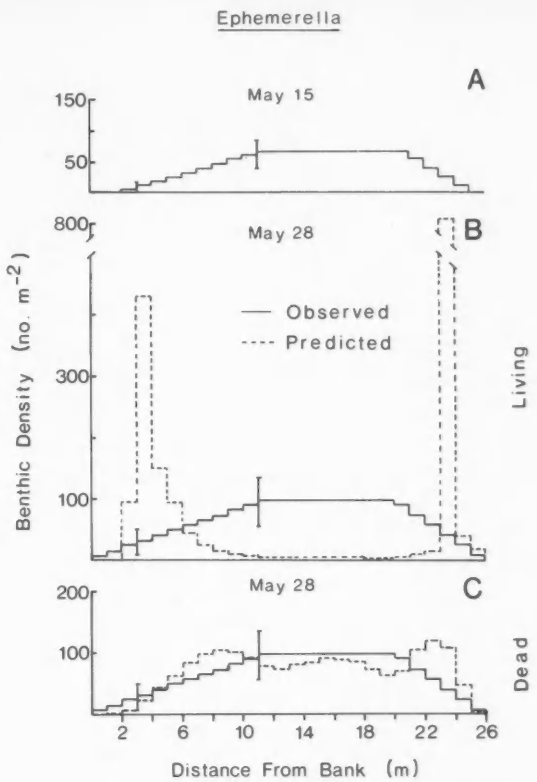

FIG. 4. Observed (solid lines) and predicted (broken lines) distribution of *E. inermis* larvae in the model river. Vertical lines represent 95% C.I. of densities of living larvae observed in the Pembina River 3 and 11 m from the south river margin. (A) 15 May; (B) 28 May (observed and predicted for living animals); (C) 28 May (observed for living animals, predicted for dead animals).

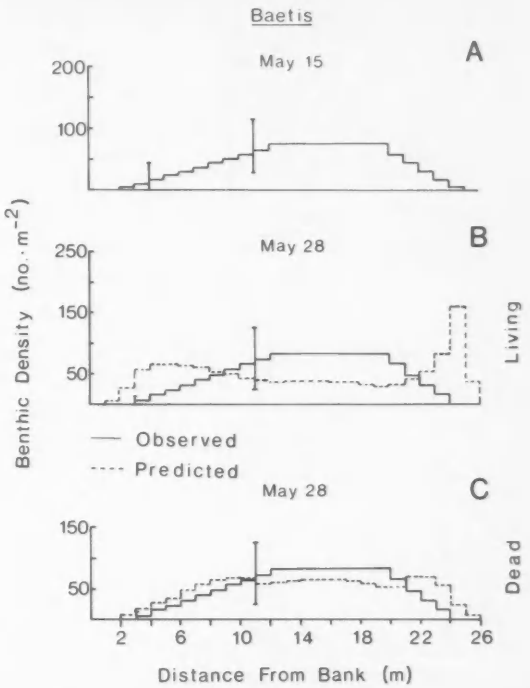

FIG. 5. Observed (solid lines) and predicted (broken lines) distribution of *B. tricaudatus* larvae in the model river. Vertical lines represent 95% C.I. of densities of living larvae observed in the Pembina River 3 and 11 m from the south river margin. (A) 15 May; (B) 28 May (observed and predicted for living animals); (C) 28 May (observed for living animals, predicted for dead animals).

to the nearest minute) were taken at 09:00, 12:00, 18:00, and 24:00. Duration of each sample varied with date and with position of a net in the river. Nets in more central regions accumulated detritus more rapidly and so were submersed for shorter periods than were more shoreward nets. All larvae caught were measured and drift was expressed as drift density (number of large animals (see "Study Site and Animals") per cubic metre of water filtered).

Drift of the two taxa is aperiodic during daylight hours but not at night (Ciborowski and Clifford 1983). Maximum drift of of *E. inermis* occurs near midnight whereas *B. tricaudatus* larvae exhibit at least two nocturnal peaks. Average daytime drift density of each population was determined by dividing the total number of large animals caught in the three daytime samples by the total volume of water filtered by each net. Midnight drift levels of *E. inermis* and *B. tricaudatus* in the Pembina River are 3.4 and 1.3 times values determined over the entire night (see Ciborowski and Clifford 1983). Accordingly, average observed nocturnal drift density was obtained by dividing numbers caught in the 24:00 sample by the volume of water filtered at that time and correcting by these conversion factors.

Predicted drift

Expected drift densities of living and dead larvae during day and night were determined using benthic distributions observed on 28 May and 4 October (Appendix 1.3). Total numbers entering the drift were estimated by multiplying the benthic density (b_i^0) by the likelihood of departure from the substrate (p_{ii}) and weighting these values by sums of proportions of individuals contributed from various upstream regions (w_{ij}).

Downstream Distance Travelled

Daily mean downstream distance travelled by living and dead animals during the first day's drift was determined for each season (Appendix 1.4). Separate estimates were generated to describe mean downstream displacement of only those animals that drifted ($\bar{x}_N$) and of the entire benthic population ($\bar{x}_B$).

Results

Benthic Distribution

Although actual benthic distributions of animals changed little over the time intervals examined, the model predicted large-scale transport of live animals towards the shore (Fig. 4–6). In all cases, predicted benthic densities of living animals fell outside the 95% confidence intervals of observed densities

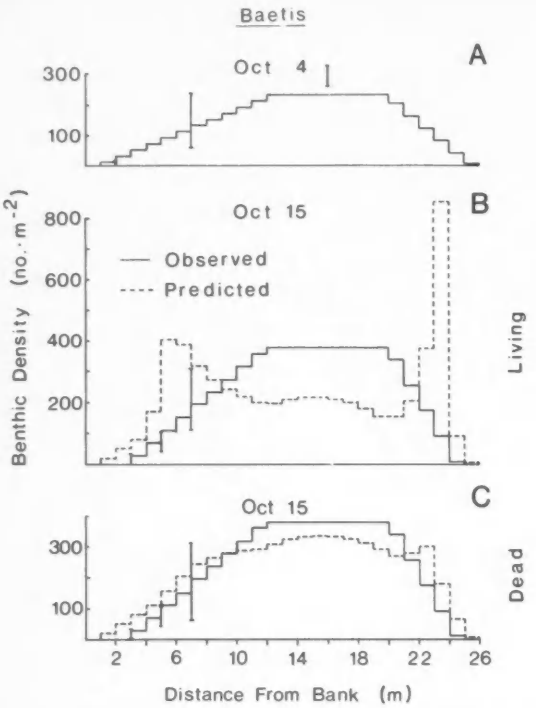

FIG. 6. Observed (solid lines) and predicted (broken lines) distribution of *B. tricaudatus* larvae in the model river. Vertical lines represent 95% C.I. of densities of living larvae observed in the Pembina River at various distances from the south river margin. (A) 4 October; (B) 15 October (observed and predicted for living animals); (C) 15 October (observed for living animals, predicted for dead animals).

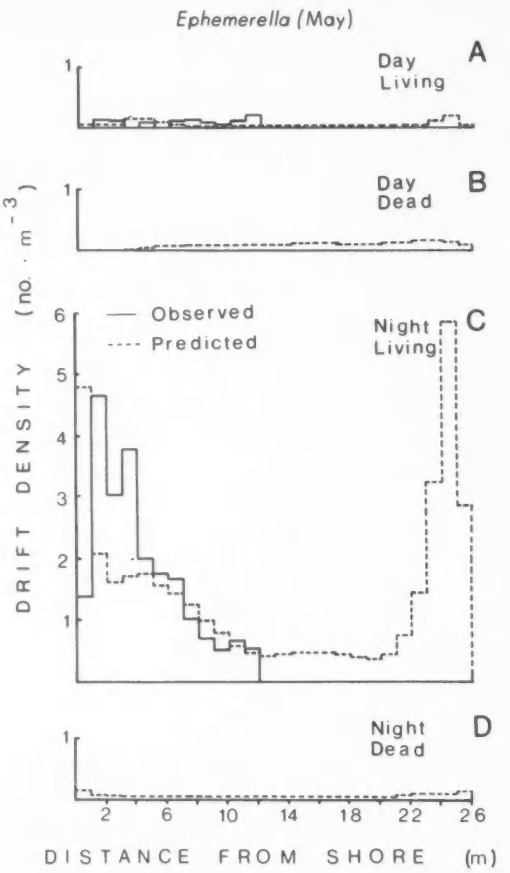

FIG. 7. Observed (solid lines) abnd predicted (broken lines) drift density of *E. inermis* larvae in May. Observed values are those recorded for living animals in regions within 12 m of the south margin of the Pembina River. Predicted values are those generated for the 26 regions of the model river. (A) living animals, observed and predicted, daytime; (B) dead animals, predicted, daytime; (C) living animals, observed and predicted, nighttime; (D) dead animals, predicted, nighttime. Note that scales of vertical axes differ.

at the 3-, 7-, and 11-m distances from shore. Model estimates also suggested that densities should become greater adjacent to the steeper north shore than the south shore.

Model-generated predictions suggested that living animals exhibit more pronounced lateral distributional changes than would their dead counterparts, due in large part to the higher propensity of the former to depart from the substrate (Fig. 2). Comparison of predictions among populations of living animals indicated that the smaller (October) *B. tricaudatus* larvae were less redistributed than the larger (May) animals (Fig. 5, 6). However, the greatest difference between expected and observed benthic distributions occurred for *E. inermis*. Transition matrices (*C*) of all populations had at least one absorbing state, corresponding to regions near shorelines. Thus, observed benthic microdistributional differences among populations after 11–13 d of drift reflect variation only in rate of accumulation in regions corresponding to these absorbing states and not necessarily differing final distributions: the model suggests that after infinite time, virtually all animals should be in regions adjacent to river margins.

Drift Density

Predicted drift densities of populations of dead animals were substantially lower than predicted drift densities of corresponding living populations except in midstream.

Observed drift densities of *E. inermis* were much greater in May than in October (Fig. 7, 8). In May, predicted drift densities of living animals grossly corresponded to values observed in the Pembina River both during day and night.

Observed nocturnal drift densities of *B. tricaudatus* larvae were slightly greater in May than in October (Fig. 9, 10). The reverse was true for daytime values. The model markedly underestimated May drift densities of living larvae. October predictions of drift density were higher than observed during daytime and lower than observed at night. All predictions of drift densities of dead animals were below the values observed for living animals in the Pembina River.

Downstream Transport Distance

Of animals predicted to depart from the substrate in the model river, dead individuals of both species were carried at least twice as far downstream as their living counterparts

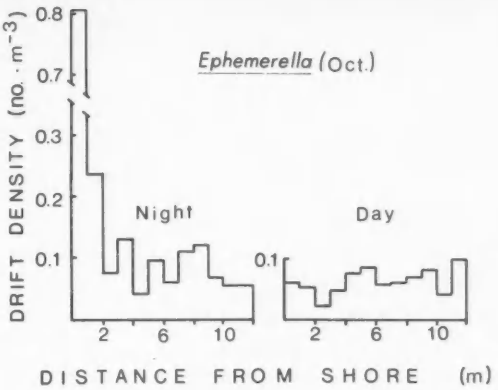

FIG. 8. Observed drift density of *E. inermis* larvae in the Pembina River in October (A) during night and (B) during day.

TABLE 3. Predicted daily mean downstream drift ($\bar{x}_N$) and net downstream displacement of populations ($\bar{x}_B$) in the model river.

Species	Condition	$\bar{x}_N$	$\bar{x}_B$
E. inermis (May)	Living	15.10	14.98
	Dead	37.29	5.59
B. tricaudatus (May)	Living	8.25	6.44
	Dead	17.70	3.24
B. tricaudatus (Oct.)	Living	8.99	6.43
	Dead	26.00	1.76

(Table 3). However, because living animals had greater overall departure tendencies (Fig. 2), daily downstream displacement of entire populations was greater for living than for dead animals; the *E. inermis* population was displaced the furthest.

Discussion

Model predictions suggest that drift should transport large numbers of both *E. inermis* and *B. tricaudatus* from the river center to nearshore regions. Sensitivity analyses of model behavior (Ciborowski 1982) indicate that the sign of the relationship between departure tendency and current velocity determines where animals will accumulate, provided that lateral dispersal coefficients are greater than zero at all points in the river. If departure is a positive function of velocity, densities increase near shore; if a negative function, greatest densities are maintained at river center. However, if lateral dispersal values near shore approach zero (i.e. water is too shallow or slow moving to permit later emigration by drift), populations will tend to accumulate and become restricted to marginal areas. In such cases, reduced departure tendencies or increased settling rates at stream center can only retard the rate of change of distribution. My estimates of lateral dispersal for living animals of both populations included at least one value near zero. Thus, if the model is valid, and animals relocate only by departing from the substrate, drift activity should eventually transport and restrict all *E. inermis* and *B. tricaudatus* larvae to river margins. Apparently, behavior that reduces departure within fast-water areas cannot compensate for the lateral transport associated with behavioral actions facilitating return to the substrate (Ciborowski 1983a). Indeed, the drift behavior of living animals results in a more pronounced lateral displacement than would occur if drift was just a mechanical phenomenon: an erosion and displacement of dead organisms. Note that many other taxa transported in the water column have minimal settling capabilities (Elliott 1971a). Because return to the substrate of such animals (e.g. Chironomidae, Simuliidae, some Trichoptera) is a function of size rather than shape (Ciborowski and Corkum 1980), their expected redistributions resulting from drift could be expressed by the transition matrices for dead animals.

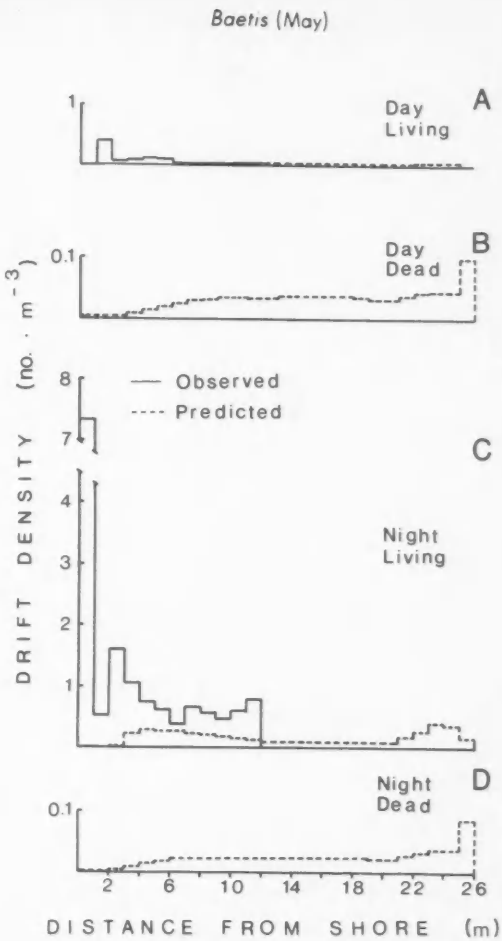

FIG. 9. Observed (solid lines) and predicted (broken lines) drift density of *B. tricaudatus* larvae in May. Observed values are those recorded for living animals in regions within 12 m of the south margin of the Pembina River. Predicted values are those generated for the 26 regions of the model river. (A) living animals, observed and predicted, daytime; (B) dead animals, predicted, daytime; (C) living animals, observed and predicted, nighttime; (D) dead animals, predicted, nighttime. Note that scales of vertical axes differ.

The model clearly fails to predict the standing benthic distribution of animals across the river. This precludes using drift alone to explain microdistribution of these rheophilic populations. Yet, the predicted magnitude and spatial patterns of drift density, which were derived from the same data, were surprisingly similar to those observed in the Pembina River.

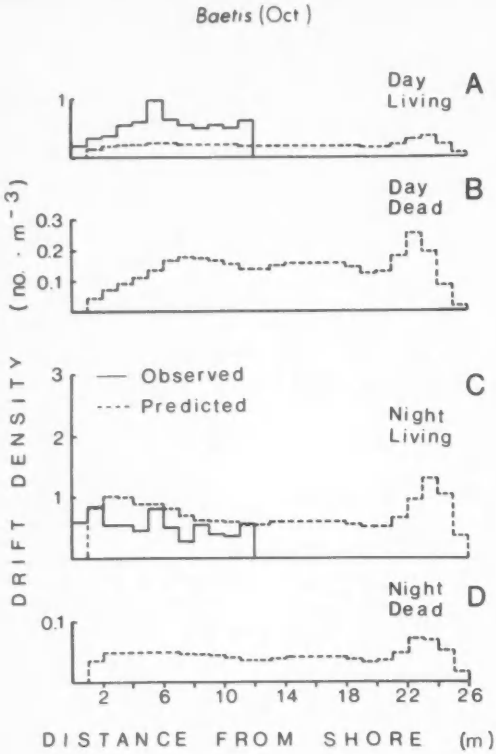

FIG. 10. Observed (solid lines) and predicted (broken lines) drift density of *B. tricaudatus* larvae in October. Observed values are those recorded for living animals in regions within 12 m of the south margin of the Pembina River. Predicted values are those generated for the 26 regions of the model river. (A) living animals, observed and predicted, daytime; (B) dead animals, predicted, daytime; (C) living animals, observed and predicted, nighttime; (D) dead animals, predicted, nighttime. Note that scales of vertical axes differ.

Where marked differences occurred, they were underestimates of observed levels. This could result from a possibly inappropriate assumption that nocturnal settling rates are equivalent to daytime settling rates (Luedtke and Brusven 1976; Walton 1978; but see Elliott 1971a), or because certain individuals engaged in multiple drift episodes. However, any significant underestimate of either numbers of animals transported or of downstream transport distance would produce a corresponding underestimate of lateral redistribution. Consequently, the changes in benthic distribution predicted by the model must describe the direction, if not the exact magnitude, of the effect of drift on microdistribution of the two mayfly populations.

If benthic patterns predicted from drift alone cannot explain benthic microdistribution of *E. inermis* and *B. tricaudatus* larvae in the Pembina River, and the estimates of drift are realistic, then these organisms must undergo some form of compensatory movement that counteracts the effects of drift. The net lateral active movement necessary to balance predicted lateral transport cannot be directly estimated because there is no unique set of probabilities of travel among regions that will exactly counteract the changes in position dictated by the transition matrices. However, I used an iterative method to generate one estimate of the approximate minimal degree of movement towards stream center necessary to balance loss of insects from the model river's eight central regions after the first day of drift (Appendix 1.5). Maximum daily net distance that would have to be travelled by dead animals starting at the south river margin (intercept of compensating function) would be between 0.3 and 1.0 m (Table 4). Maximum net distances calculated for living *B. tricaudatus* and *E. inermis* larvae were 2–3 and 6.7 m, respectively.

TABLE 4. Functions describing estimated mean lateral benthic travel distance (g_i) that must be traversed by animals presently in each region (i; $i \leq 12$) to compensate for previous lateral transport by drift in the model river. Equations take the form $g_i = \gamma_0 + \gamma_1 y_i$ where y_i is distance from the south river margin.

Species	Condition	Intercept γ_0	Slope γ_1
E. inermis (May)	Living	6.7	−0.57
	Dead	1.0	−0.08
B. tricaudatus (May)	Living	2.9	−0.24
	Dead	0.7	−0.05
B. tricaudatus (Oct.)	Living	2.1	−0.17
	Dead	0.3	−0.02

Movements of this magnitude are probably within the capabilities of larvae of these species. Elliott (1971b) estimated than *B. rhodani* (Pictet) and *E. ignita* (Poda) larvae travelled daily modal upstream distances of 6.25 and 4.75 m in a field-situated running water trough. Although lateral movements in his source stream were not as pronounced as those upstream, Elliott (1971b) trapped many more animals crawling towards the center than towards the margins. In contrast, Bird and Hynes (1981) reported random benthic movements of invertebrates in an Ontario river.

Seasonal data on benthic distribution of *E. inermis* larvae in the Pembina River (Ciborowski and Clifford 1983) indicate that benthic movements do carry large numbers of animals away from margins at certain times. Newly hatched larvae predominate near margins in late summer, but by mid-October, densities have become greatest in regions remote from shore. Because drift is reduced in autumn (Fig. 8), this redistribution can occur only through benthic movement. Seasonal net offshore movements of lentic mayfly populations have been reported by several investigators (Gibbs 1979; Corkum 1984; J. E. Brittain, University of Oslo, pers. comm.).

Changes in benthic microdistribution in the Pembina River during and after flood episodes are also consistent with model implications. *Baetis tricaudatus* and *E. inermis* larvae become more concentrated near shore, and densities further from margins become reduced (Ciborowski and Clifford 1983). Because benthic invertebrate movement is inhibited by high current velocities (Elliott 1971b), increased discharge associated with flooding would both accelerate lateral transport and restrict larvae to relatively slow-water marginal areas. Both effects would result from an increased incidence of (catastrophic) drift due to scouring as well as animals' inability to regain more central regions. Only after water levels recede could animals again return to the more central regions. This microdistributional pattern has also been reported for other mayflies in an Oregon stream (Lehmkuhl and Anderson 1972).

If my hypothesis that frequent participation in drift requires a coincident ability to engage in extensive lateral benthic

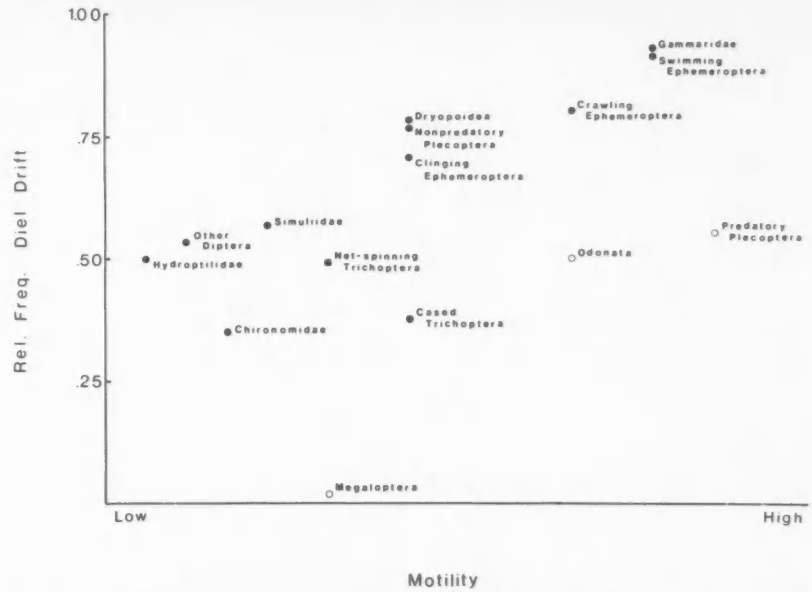

FIG. 11. Relationship between nondrift motility (subjective ranking) and relative frequency of reports of nocturnal periodicity in drift among 15 behavioral/taxonomic groups of lotic invertebrates. Solid circles represent nonpredatory organisms; open circles represent predatory organisms.

movement is valid, one would expect to find less pronounced drift among sessile organisms than among vagile animals. To evaluate this, I subjectively ranked the locomotory ability of 15 behavioral/taxonomic groups of aquatic invertebrates. I then searched the literature for reports of drift periodicity of these groups (Appendix 1.6). My measure of behavioral drift activity was the relative frequency of citations for which diel (night-active) periodicity was observed. Two trends were evident (Fig. 11). Drift periodicity of nonpredatory taxa was more prevalent than that of predatory organisms; and frequency with which diel drift periodicity occurs was positively correlated with benthic locomotory ability (Spearman's rank correlation coefficient = 0.57, $p < 0.05$ (all taxa included), and 0.78, $p < 0.01$ (nonpredatory taxa only)).

Drift has traditionally been viewed as an accidental consequence of foraging and/or competition that permanently removes individuals from their immediate surroundings (Gyselman 1980). This is suggested to result in either colonization of unexploited downstream reaches (Müller 1954, 1973, 1974) or use of the excess production (Waters 1965, 1972) by other trophic levels (drift-feeding predators). Both of these hypotheses have received qualified support. Reports of large-scale drift of early instars of aquatic taxa (Waters 1972; Müller 1974; Colbo and Moorehouse 1979) are consistent with Müller's hypothesis. Although density-dependent drift responses of older animals, which support Water's hypothesis, have been observed in certain circumstances for some taxa (Wiley 1981; Statzner et al. 1984), unequivocal results for free-ranging organisms have seldom been demonstrated. Among vagile Ephemeroptera and nonpredatory Plecoptera, most controlled studies indicate that predator activity (Corkum and Pointing 1979; Corkum and Clifford 1980; Peckarsky 1980; Walton 1980), local food depletion (Hildebrand 1974; Keller 1976; Bohle 1978; Kohler 1985), and current velocity (Corkum et al. 1977; Ciborowski 1983b) all supersede competitive interactions as factors inducing drift.

It is significant that those taxa for which density-dependent drift has been implied to occur typically rely upon a fixed area of substrate to fulfill spatial or feeding requirements, and are coincidentally aggressive toward intruders (net-spinning caddisflies, Glass and Bovbjerg 1969; Hildrew and Townsend 1980; Mogel et al. 1985; Hydroptilidae, Hart 1983; McAuliffe 1983; Simuliidae, Harding and Colbo 1981; Wiley and Kohler 1981; Chironomidae, Wiley 1981). Furthermore, the morphology of these animals is not conducive to efficient large-scale benthic movement. Although these organisms do occasionally drift in large numbers, they do not exhibit the marked periodicity characteristic of other taxa. In contrast, taxa that do not show density-dependent drift are mobile grazing or leaf shredding animals. Interactions involving these organisms elicit short-scale benthic movement, but not drift (Corkum and Clifford 1980; Wiley and Kohler 1981; Statzner and Mogel 1985). Drift of the study taxa in the Pembina River is so pronounced that loss of these animals would decimate populations within the central riverbed within several days. Consequently, I suggest that drift of such larvae results in only local displacement, and is thus an adjunct to normal benthic activity. The population turnover rates implied by recent colonization studies suggest that this is not a unique phenomenon (Sheldon 1984).

Yet drift cannot serve as a local relocation mechanism independently of active benthic movement. Drifting animals are probably incapable of continuously monitoring the quality of substrate over which they pass, and tend to be carried to less suitable (slow-water) regions. The model indicates that these areas can only be left by active means; this would nullify any possible energetic savings of drift over benthic movement. Presumably, a mobile herbivore should drift only if the alter-

native is mortality (predator attack) or if prior active movement fails to carry it into a suitable microhabitat (adequate food, substrate, or current). Largely unidirectional movement is the most efficient means of locating randomly distributed resources (Pyke 1978), and this should be even more the case where resources are longitudinally stratified (i.e. more abundant in some regions (sensu the model) than others). Kohler (1984, 1985) has recently related directionality and degree of movement of *Baetis* larvae to periphyton levels. Periphyton abundance is frequently positively associated with specific current velocity levels in rivers (Horner and Welch 1981). Rheophilic animals, whether drift prone or not, would benefit from active movement that directs them into faster water. Subsequent lateral transport by drift, however, may act to transport unsuccessful animals to a point (near stream margin) from which further lateral benthic movement would carry them centerward across the entire gradient of available microhabitats. In such instances, drift would have provided an energetically efficient means of sampling at least one new microhabitat (the point of settling) and without necessarily having caused large net downstream or lateral displacement relative to a premovement position. This might be particularly important where the relative position of resources changes through time in concert with daily or seasonal variations in flow pattern or discharge.

Because drift represents a generalized response to many factors, it is not surprising that no single hypothesis can adequately describe its ecological significance among all aquatic taxa. Although the hypotheses of Müller and Waters do pertain to certain organisms at some times, they are not universally applicable. Implications of the drift model regarding dynamics of transport provide some predictive value in anticipating the role of drift among different aquatic groups. High intensity, possibly endogenous seasonal drift of early instars of several aquatic taxa (Baetidae, some Trichoptera, Simuliidae) probably entails permanent downstream transport. Although this may produce significant mortality, it permits successful colonization of unexploited downstream reaches (distributional drift model of Müller (1973)). Among larger rheophilic animals that have poor locomotory ability and depend on being in suitable feeding locations, drift may reflect the outcome of aggressive interactions. Drifting animals of these populations may indeed be lost from the system, since they lack the locomotory capacity to regain more favorable areas. These organisms behave in accordance with Waters' limited production model. I propose that among larger free-ranging taxa, drift is a complement to benthic search behavior, serving to transport and relocate animals from unfavorable regions to either more suitable areas or to a "starting point" from which further search may be initiated.

Note that because the model was developed for a large, spatially stratified system, this latter interpretation of the role of drift may not apply as well to small streams in which microtopography (snags, boulders) may produce a mosaic of microhabitats. Although the dynamics of departure and transport should still pertain as presented here, downstream variability in settling rates and lateral transport could induce quite different microdistributional patterns of larvae. Furthermore, the dimensions of small streams may be such that free-ranging individuals could sample the entire range of microhabitats available solely through relatively short-scale benthic movement. In such an instance, drift may assume subsidiary importance. Incidence of drift (drift density) in smaller systems is typically substantially less than that observed in the Pembina River (see Armitage 1977). This raises the possibility that the behavioral repertoire governing locomotion of vagile aquatic invertebrates might be regulated among lotic systems to coincide with the spatial arrangement of suitable microhabitats rather than simply to the extensiveness of those microhabitats.

In summary, drift of *E. inermis* and *B. tricaudatus* larvae in the Pembina River is of great enough magnitude to produce profound changes in benthic microdistribution of populations over short time intervals. Short-term stability in distribution of these populations appears to be maintained despite the propensity of animals to drift. Because drift provides access largely to slower portions of the river, active movement is a necessary corequisite to permit exploitation of all regions of the lotic system. An individual that can relocate by drifting and continue benthic search may have an increased likelihood of encountering stratified, resource-rich areas. In contrast, benthic organisms with poor locomotory ability may be relegated to unfavorable marginal areas. Internally or externally mediated changes in the tendency of individuals to drift or to engage in active lateral movements should result in an alteration of regions of population concentration.

Acknowledgments

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Appendix

1.1 List of Symbols

Lowercase letters represent scalar quantities. When subscripted, they represent elements of vectors or matrices. Uppercase letters represent vectors. Boldfaced uppercase letters represent matrices.

Symbol	Description
B^t	Vector of benthic density of animals in regions of model river on day t
C^t	tth power of transition matrix describing probability of change in benthic position of an animal among regions of model river
D	Number of hours of darkness
$F(x)$	Vector of proportion of suspended animals that settle over a unit of downstream distance, x metres from a point of entry into the water column in regions of the model river
I, I	Identity matrix, vector
K	Matrix representing probability that an animal suspended in the water column a given region settles in a given region of the model river
L	Number of hours of daylight

$M(x)$ Vector of proportion of animals suspended in the water column over unit length of regions, x metres downstream of a point of entry into the water column

$N(x)$ Vector of number of animals suspended in the water column over unit length of regions, x metres downstream of a point of entry into the water column

$N_{max}(t)$ Vector of total number of suspended animals that pass a line transecting regions during time interval t

$P(t)$ Diagonal matrix representing proportion of animals that depart from substrate of regions during time interval t

$Q(t)$ Diagonal matrix representing proportion of animals that remain on substrate of regions during time interval t

R Vector of instantaneous settling rate of suspended animals in regions of model river

S Vector of measure of lateral diffusion of suspended animals (standard deviation units) in regions of model river

t Time

V Vector of mean current velocity in regions in of model river

W Matrix representing sums of proportions of animals suspended in the water column following departure from a number of points upstream in regions

x Downstream distance travelled by an animal suspended in the water column

$\bar{X}$ Vector of mean daily downstream distance travelled by all animals that depart from the substrate in regions in the model river

$\bar{\bar{x}}_N$ Mean daily downstream distance travelled by all animals that depart from substrate in model river

$\bar{\bar{x}}_B$ Mean daily downstream displacement by drift of entire population in the model river

Y Vector of distance of midpoint of model river regions from shore

Z Vector of mean depth of regions in model river

α Regression coefficient

β Regression coefficient

1.2 Calculation of Matrices K and W

Probabilities of transport of animals from one region to all others were estimated using equations 7 and 8 (see also Ciborowski 1982). Proportions of animals settling at increasing 1-m steps downstream of a given entry point, y_i, into the water column were determined from equation 6. The proportion not settling $(m(x)_i)$ at each step was partitioned according to a normal probability distribution generated around a mean (y_i) and a standard deviation (s_i). Proportions returning to the substrate at each step were summed until the total fraction that had settled exceeded 0.999. The remainder was added to the region from which animals originated. Summing the proportions of animals suspended in each region at the end of each step $(m(x)_i)$ provided an estimate, w_{ij}, of the contribution of animals originating in each region i to the drift at other regions, j. Documentation for and copies of computer programs (written in APL) used to generate these data and other model results are available from the author.

1.3 Estimation of Predicted Drift

The expected total number of animals, n_i, departing from 1 m^2 of substrate in region i of the model river during day (L) and at night (D) are

$$(9) \quad n_i(L) = b_i p(L)_{ii}$$

$$(10) \quad n_i(D) = b_i p(D)_{ii}$$

where b_i is the benthic density and p_{ii} is the proportion that depart. However, because the concentration of animals in the water column above each square metre of each region also depends on water depth (z_i), the concentration in each region is

$$(11) \quad n_i'(L) = n_i(L)/z_i$$

$$(12) \quad n_i'(D) = n_i(D)/z_i.$$

Since matrix W represents the sum of proportions of animals departing from region i that would be carried across a line in region j, the total expected number of animals caught in drift nets placed across the regions during the day $(N_{max}(L))$ and at night $(N_{max}(D))$ would be

$$(13) \quad N_{max}(L) = N(L)W$$

$$(14) \quad N_{max}(D) = N(D)W.$$

Predicted drift density in each region was estimated by dividing $N_{max}(L)$ and $N_{max}(D)$ by the volumes of water expected to pass through drift nets (determined from Fig. 1) during day and night, respectively.

1.4 Downstream Distance Travelled

The sum of proportions of animals that drift past a line across a river is the product of the proportion departing from substrates upstream and mean distance travelled (Elliott 1971a). Thus,

$$(15) \quad w = m(0)x.$$

When $m(0) = 1.0$, as is the initial condition in estimating values for matrices K and W, this reduces to

$$(16) \quad x = w.$$

Since W is the matrix of sums of proportions of suspended animals transported from any region i to each region j, the mean downstream distance travelled by animals departing from the substrate in any region $(\bar{x}_i)$ is the ith row total of W, i.e.

$$(17) \quad \bar{x}_i = w_{i1} + w_{i2} + \ldots + w_{i26}$$

$$\bar{X} = (x_1, x_2 \ldots x_{26}).$$

The mean distance travelled by all animals that drift during 1 d $(\bar{\bar{x}}_N)$ is

$$(18) \quad \bar{\bar{x}}_N = N(24)\,\bar{X}/N(24)I$$

where $N(24)$ is the vector of the number of animals that depart from the substrate over 24 h and I is the identity vector (a vector with all elements consisting of ones). Mean daily downstream displacement of the entire population resulting from drift is

$$(19) \quad \bar{\bar{x}}_B = N(24)\bar{X}/B'I$$

where B' is the vector of benthic density of animals on day t. Note that because $n(24)_i = b_i p(24)_{ii}$, both $\bar{\bar{x}}_N$ and $\bar{\bar{x}}_B$ depend on benthic distribution and consequently vary as this changes.

1.5. Interpolated Benthic Distances Moved

To estimate lateral benthic movement necessary to balance drift, I assumed that activity (thus, net distance that would be travelled) was a negative linear function of mean current velocity (hence initial distance from shore) (see Edington 1965; Elliott 1968) and that animals either remained in their present region or moved towards stream center. Net movement was arbitrarily presumed to be nondirectional (zero) in central regions and 0.1 $m \cdot d^{-1}$ in regions immediately adjacent to center.

Probability of movement from any particular region to a more central region was assigned according to a Poisson series. The first iterative step involved selecting an arbitrary intercept value for the relationship between initial distance from shore (south margin) and mean net lateral distance moved. The slope necessary to produce mean lateral travel of 0.1 $m \cdot d^{-1}$ adjacent to the center was then determined and the mean distance moved by animals within all other regions interpolated from this linear function. Probability of lateral movement by any animal from a given region to each other region was calculated according to the Poisson distribution corresponding to the interpolated mean distance travelled from that region. Probabilities were then assembled as a transition matric, C'. The inner product of this matrix and the vector describing benthic distribution after 1 d of drift (B^1) was then calculated:

$$(20) \quad B^{0\prime} = B^1 C'.$$

The elements of $B^{0\prime}$ corresponding to densities in the model river's eight central regions were then summed. The function describing active movement was judged adequate to compensate for lateral transport by drift if the sum above equalled or exceeded the corresponding sum of elements of B^0. If the function was inadequate, a new (greater) intercept was selected, a corresponding slope determined, and a new transition matrix derived and evaluated.

1.6. Literature Survey of Diel Periodicity

Citations were taken from field studies that addressed day–night drift variations of at least one taxon (63 papers). A citation consisted of a verbal, tabular, or figured description of one taxon appearing in the drift at one stream site on one date in amounts great enough for periodicity to be potentially discerned. Where drift was repeatedly sampled, multiple citations were admitted for each sample at least 1 mo removed from other collections, to a maximum of 12. If samples were taken throughout a year and only a general statement of pattern was made, I assigned up to four citations (one per season). Where patterns were described for specific developmental stages, periodicity was assessed on the basis of drift of all stages pooled over one date.

Relative frequency was calculated by summing all instances of night-active periodicity for a group and dividing by all citations for that group. Total number of citations and the number of papers on which they were based is as follows: Gammaridae (Amphipoda) 14 (10 papers); Odonata 2 (2); swimming Ephemeroptera 101 (36); crawling Ephemeroptera 77 (24); clinging Ephemeroptera 54 (21); predaceous Plecoptera (Systellognatha) 38 (12); herbivorous Plecoptera (Holognatha) 56 (15); Megaloptera 2 (2); Dryopoidea (Coleoptera) 37 (10); net-spinning Trichoptera 61 (15); Hydroptilidae (Trichoptera) 19 (4); case-building Trichoptera 56 (15); Chironomidae (Diptera) 114 (24); Simuliidae 65 (19); other Diptera 45 (14). A bibliography of papers from which citations were taken is available from the author.

Thermally Modulated Biliary Excretion of [^{14}C]Taurocholate in Rainbow Trout (*Salmo gairdneri*) and the Na^+,K^+-ATPase[1]

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Kemp, C. J., and L. R. Curtis. 1987. Thermally modulated biliary excretion of [^{14}C]taurocholate in rainbow trout (*Salmo gairdneri*) and the Na^+,K^+-ATPase. Can. J. Fish. Aquat. Sci. 44: 846–851.

Biliary excretion rate of [^{14}C]taurocholate was 53–63% greater in rainbow trout (*Salmo gairdneri*) acclimated to and tested at 18 than 14 or 10°C. Acute 4° temperature shifts up or down increased or decreased respectively biliary excretion rate by 40–53%. Furthermore, 10°C acclimated fish shifted to 14°C had 125% greater biliary excretion rate than 18°C acclimated fish shifted to 14°C. Apparent hepatic blood flow was greater in fish acclimated to and measured at 18 than 14 or 10°C but was not different when 10 or 18°C acclimated fish were measured at 14°C. Acclimation to 10 versus 18°C did not affect the V_{max} or the temperature/activity relationship for the liver plasma membrane (LPM) Na^+,K^+-ATPase or the LPM Mg^{2+}-ATPase but did cause a major shift in the K_m of the Na^+,K^+-ATPase (0.14 and 1.11 m*M*, respectively). This K_m shift was observed at an 18°C but not a 37°C assay temperature. Thus, acclimation-induced changes in this enzyme were only observable at physiologically relevant temperature and substrate conditions. Depending on the thermal treatment, both hepatic blood flow and LMP Na^+,K^+-ATPase may module biliary excretion.

Le taux d'excrétion biliaire du taurocholate-[C^{14}] était de 53 à 63 % plus élevé à 18°C qu'à 14 ou 10°C chez la truite arc-en-ciel (*Salmo gairdneri*) acclimatée et testée à ces températures. Une augmentation ou une diminution brutales de 4°C ont entraîné respectivement une augmentation et une diminution du taux d'excrétion biliaire qui ont varié de 40 à 53 %. De plus, les poissons acclimatés à 10°C et introduits dans un milieu à 14°C montraient un taux d'excrétion biliaire de 125 % plus élevé que les poissons acclimatés à 18°C et transférés dans un milieu à 14°C. Le débit évident du sang hépathique était plus élevé chez les poissons acclimatés et testés à 18°C que chez ceux acclimatés à 14 ou 10°C; par contre, il n'était pas différent chez les poissons acclimatés à 10°C ou 18°C et testés à 14°C. L'acclimatation à 10°C versus 18°C n'a pas influé sur la V_{max} ni sur la relation température/activité du Na^+, de la K^+-ATPase ou de la Mg^{+2}-ATPase de la membrane plasmatique hépathique, mais a entraîné une variation importante du K_m du Na^+ et de la K^+-ATPase (0,14 et 1,11 m*M* respectivement). Cette variation du K_m a été observée à une température expérimentale de 18°C mais non à 37°C. Ainsi, les variations de cet enzyme provoquées par l'acclimatation ne pouvaient être observées qu'en présence de températures et de substrats physiologiquement pertinents. Selon la température, le débit sanguin hépathique et les concentrations de Na^+ et de K^+-ATPase dans la membrane plasmatique hépathique peuvent moduler l'excrétion biliaire.

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Environmental temperature affects elimination of xenobiotics from tissues of poikilotherms. Increased environmental temperature stimulated elimination of naphthalene from flounder (*Platichthys stellatus*) (Varanasi et al. 1981), hexachlorobenzene and mirex from rainbow trout (*Salmo gairdneri*) (Niimi and Palazzo 1985), and benzo[a]pyrene from spiny lobster (*Panulirus argus*) (Little et al. 1985). Such differences were not readily explained in terms of altered biotransformation rates. Further, studies on rainbow trout indicated that in vivo glucuronidation (Curtis 1983) and in vitro cytochrome P-450 mediated oxidations (Koivusaari et al. 1981) exhibited "ideal temperature compensation." These results likely reflected temperature acclimation, an adaptive mechanism whereby poikilotherms maintain essential life processes across a range of environmental temperatures (Hazel and Prosser 1974; White and Somero 1982).

Biliary excretion of exogenously provided organic anions by rainbow trout was stimulated at warmer environmental temperatures and was proposed as an important factor in reduced xenobiotic half-life in poikilotherms (Curtis 1983; Curtis et al. 1986). Here, we examine whether temperature (both immediate and long term) modulated the state of the hepatobiliary system and describe potential mechanisms for such modulation. We measured in vivo biliary excretion rate of [^{14}C]-taurocholate, hepatic blood flow, and in vitro temperature/activity relationships and kinetics of liver plasma membrane (LPM)ATPases from thermally acclimated rainbow trout. In vivo parameters were measured at acclimation temperatures (10, 14, and 18°C) and after acute 4° changes in environmental temperature. In vitro kinetics and temperature/activity profiles of LPM Na^+,K^+- and oligomycin-insensitive Mg^{2+}-ATPase were determined to assess the potential role of these enzymes in thermally modulated hepatobiliary performance. The Na^+,K^+-ATPase utilizes ATP to produce Na^+ and K^+ grad-

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ients across the hepatocyte plasma membrane, the energy of which is used to transport organic anions (e.g. taurocholate) from plasma to hepatocyte (Scharschmidt and Stephens 1981; Blitzer et al. 1982). While the LPM Mg^{2+}-ATPase is clearly associated with the biliary surface of the hepatocyte (Graf et al. 1984), its physiologic role is not established.

Materials and Methods

Animals and Materials

Shasta strain rainbow trout of both sexes (200–500 g) were acclimated for 4 wk to 10, 14, or 18°C as previously described (Curtis et al. 1986). Analytical grade reagents were used throughout. Oligomycin (65%A, 20%B, 15%C), Tris–ATP, ouabain, MS 222 (tricaine methanesulfonate), and the sodium salt of taurocholate (98% pure) were purchased from Sigma Chemical Co. (St. Louis, MO). Tissue solubilizer (Fisher Scintigest) and scintillation cocktail (Scintiverse I) were from Fisher Scientific Co. (Santa Clara, CA), and [^{14}C]taurocholate (specific activity 1.2 mCi·mmol^{-1} (44 MBq·mmol^{-1}), carbonyl-^{14}C) was obtained from California Bionuclear (Sun Valley, CA).

[^{14}C]Taurocholate was mixed with unlabeled taurocholate to yield a 10 m*M* solution (2.4 μCi·mL^{-1} (88.8 kBq·mL^{-1})) in distilled water. Purity of this stock and biliary [^{14}C]taurocholate was verified by thin-layer chromatography (Eneroth 1963) using silica gel plates and a solvent system of butanol–acetic acid–water at a ratio of 85:10:5.

Biliary Excretion of Taurocholate

Biliary excretion rate was measured essentially as described (Curtis et al. 1986). Thermally acclimated fish (10, 14, and 18°C) were starved for 48 h, injected with [^{14}C]taurocholate (2.4 μCi·kg^{-1} (88.8 kBq·kg^{-1}) i.p.), and transferred to static aerated aquaria. At 60 min, the fish were sacrificed and the liver and gallbladder removed, separated, and weighed. Duplicate 0.1-g samples of bile were pipetted into scintillation vials and digested for 24 h in 1 mL of tissue solubilizer and 0.2 mL of water. Nine millilitres of scintillation cocktail was added and the samples counted in a scintillation counter. Some fish were subjected to acute 4° shifts in body temperature by transferring them to the appropriate aquaria 1 h prior to and during the experiment. Biliary excretion rate was measured at 60 min after taurocholate administration because excretion rate was linear and biliary retention was maximal at this time (Curtis et al. 1986 and unpublished results).

Hepatic Blood Flow

Hepatic blood flow was measured by laser–Doppler blood flowmetry (LDBF) with a Med Pacific LD5000 instrument equipped with a fiberoptic probe (Seattle, WA). Laser light was conducted via one fiber up to 1 mm into the tissue. This light, deflected back by moving red blood cells, was received and transmitted via a second fiber to be electronically processed to a signal in millivolts (Bonner and Nossal 1981). We examined hepatic blood flow in fish immobilized by spinal transection to avoid effects of anesthesia. Curtis et al. (1986) demonstrated that elimination of i.p.-administered [^{14}C]taurocholate was similarly affected by environmental temperature in free-swimming and spinally transected rainbow trout. Fish were thermally acclimated as above, spinally transected at the distal margin of the opercula (Schmidt and Weber 1973), placed in a Plexiglas frame to maintain upright body position, and allowed to stabilize for 24 h at their acclimation temperature. Animals were then positioned ventral side up in the frame in a temperature-controlled water bath. A medial incision was made to expose the liver, and blood flow measurements were first made at the acclimation temperature (10, 14, or 18°C) and after 4° changes in body temperature. Great care was taken to maintain the proper optical coupling between the fiberoptic probe and liver surface. A minimum of 10 measurements were taken at different lateral and medial sites on each liver. The mean signal at these two surfaces of the liver was not statistically different and so the data were combined for analysis. Measurements made on visible blood vessels were significantly higher than the surrounding tissue and were omitted from analysis.

Membrane Preparation

A modification of the method of Lutz (1973) was used for the isolation of LPM. Thermally acclimated fish (10 and 18°C) were killed by a blow to the head and the liver excised. The gallbladder and adherent connective tissue were dissected off and 8 g of liver finely minced with scissors and a razor blade in ice-cold buffer (10% sucrose and 50 m*M* Tris, pH 7.4). This preparation was brought to 10 mL with buffer, homogenized with two strokes of a loose fitting pestle in a Dounce homogenizer, diluted to 150 mL with buffer, and centrifuged at 120 × *g* for 6 min. The supernatant and erythrocyte layer were removed by aspiration, the pellet resuspended in 30 mL of buffer by two strokes in the Dounce homogenizer, and the suspension filtered through 1-mm nylon mesh. The filtrate was diluted to 150 mL, centrifuged as before, and the pellet vigorously homogenized with 15 strokes of the pestle in 45 mL of buffer. For the majority of enzyme characterization, this preparation was centrifuged at 10 000 × *g* for 30 min and the pellet resuspended in 5–10 mL of ATPase buffer (0.3 *M* sucrose, 0.02 *M* EDTA, and 0.1 *M* imidazole, pH 7.2) to constitute an initial LPM suspension (LPM-1) which was stored at −25°C until used. The high variability of Mg^{2+}-ATPase activity among different preparations prompted us to further enrich LPM using a discontinuous sucrose gradient to yield LPM-2. Twenty millilitres of LPM-1 was layered onto discontinuous sucrose gradients consisting of 5 mL of 38% sucrose and 8 mL of 33% sucrose (w/w) in 50 m*M* Tris–HCl, pH 7.4. The gradients were centrifuged for 1 h in a Sorvall RC-5B centrifuge with a SV-288 vertical head rotor at 20 000 × *g*. The LPM-2 bands were collected from the 10–33% sucrose interface with a pipette, pelleted at 10 000 × g for 30 min, resuspended in ATPase buffer, snap frozen in liquid nitrogen, and stored at −25°C until used.

The purity of LPM-1 and LPM-2 was determined by a series of marker enzyme assays. The ratios of the activities of 5′-nucleotidase, glucose-6-phosphatase, and succinate dehydrogenase in preparations to those in the crude homogenate indicated enrichment or contamination of LPM, microsomes, and mitochondria, respectively. Assay conditions were previously reported (Lutz 1973; Curtis and Mehendale 1981). Protein was quantified by the method of Bradford (1976).

Initial difficulties were encountered in detecting consistent Na^+,K^+-ATPase activity; therefore, sodium deoxycholate pretreatment was used to activate this enzyme (Jorgenson and Skou 1970). This treatment was only used on LPM-1. Five

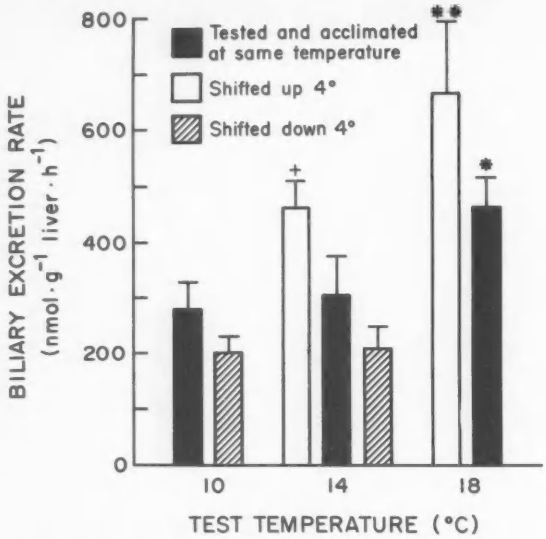

FIG. 1. Biliary excretion rate of [^{14}C]taurocholate in free-swimming rainbow trout for seven different thermal treatments. Rate is expressed as total nanomoles of [^{14}C]taurocholate equivalents in gallbladder bile per gram of liver at 1 h. Vertical bars represent + 1 SE. The Kruskal–Wallis test (Conover 1980) was used for the following groups: *acclimated and tested at 18°C (N = 10) significantly higher than 10°C at 10°C (N = 8) and 14°C at 14°C (N = 5) (solid bars); **acclimated at 14°C and shifted to 18°C (N = 3, open bar) significantly higher than 14°C at 14°C; +acclimated at 10°C and shifted to 14°C (N = 3, open bar) significantly higher than 18°C shifted to 14°C (N = 3, hatched bar).

millilitres of LPM-1 was added to 60 mL of a solution of 0.6 mg·sodium deoxycholate·mL^{-1} and 25 mM imidazole, pH 7.1, and gently stirred for 30 min at 22°C. This material was centrifuged at 10 000 × g for 30 min and the pellet brought up to 5 mL in ATPase buffer. This treatment resulted in a greater than 50% loss of protein but a five fold increase in Na^+,K^+-ATPase activity. ATPase activity was determined by adding 0.1 mL (20–50 μg of protein) to 0.2 mL of salts or inhibitors (see below), incubating for 5 min at the reaction temperature, and then adding 0.2 mL of a solution of 3.0 mM vanadium-free Tris–ATP, 3 mM $MgCl_2$, and 50 mM imidazole, pH 7.4, to start the reaction. The reaction was terminated after 15 min with 0.25 mL of ice-cold 30% TCA and the inorganic phosphate released determined by the method of Fiske and Subbarow (1925). To inhibit potentially contaminating mitochondrial ATPase, 0.5 μL of oligomycin in ethanol was initially added to each tube to give a final reaction concentration of 15 μM. Total ATPase activity was determined with 100 mM NaCl and 10 mM KCl added as salts. The Mg^{2+}-ATPase activity was the activity remaining with the substitution of Na^+ and K^+ with 110 mM choline chloride and 0.5 mM ouabain, and Na^+,K^+-ATPase was calculated as the difference between these two activities.

Statistics

Data were analyzed by one-way analysis of variance and the Kruskal-Wallis method based on ranks (Conover 1980). Significance was set at the $P \leq 0.05$ level.

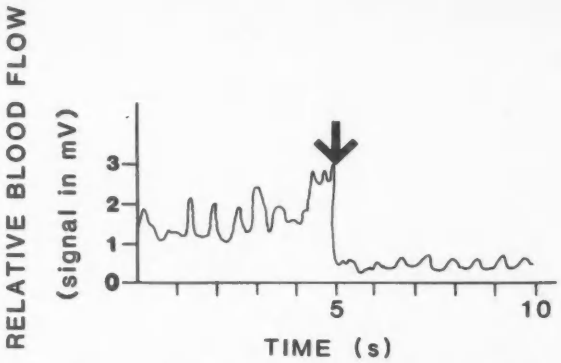

FIG. 2. Representative tracing of LDBF on the surface of trout liver before and after (arrow) portal vein occlusion. The transient shift in the baseline immediately preceding occlusion reflects the mechanical disturbance associated with the procedure.

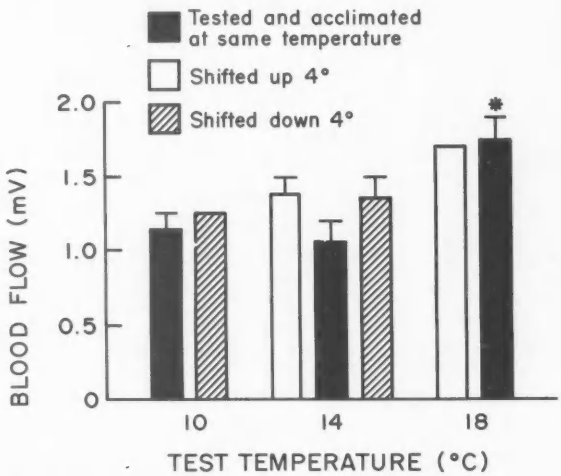

FIG. 3. Hepatic blood flow (means + 1 SE) as a function of temperature in spinally transected trout. *Acclimated and tested at 18°C significantly higher than 10°C at 10°C and 14°C at 14°C using the Kurskal–Wallis test (N = 3–4, solid bars). A single 14°C acclimated fish was shifted to 10°C (hatched bar) and another to 18°C (open bar). Three 10°C acclimated fish were shifted to 14°C (open bars) and three 18°C acclimated fish to 14°C (hatched bar).

Results

Biliary Excretion of Taurocholate

Distribution of [^{14}C]taurocholate to tissues other than liver and bile was negligible and not affected by the different acclimation and test temperatures (data not shown and Curtis et al. 1986). Biliary excretion rate of [^{14}C]taurocholate was affected by both acclimation temperature and acute temperature shifts (Fig. 1). Fish acclimated and tested at 18°C had a 53–63% higher excretory rate (463 nmol·g^{-1}·h^{-1}) than those acclimated and tested at 14°C (302 nmol·g^{-1}·h^{-1}) and 10°C (284 nmol·g^{-1}·h^{-1}). Fish acclimated at 14°C and shifted to 18°C had a significantly higher rate (666 nmol·g^{-1}·h^{-1}) than those tested at 14°C (302 nmol·g^{-1}·h^{-1}) and those shifted

down to 10°C (199 nmol·g^{-1}·h^{-1}). Acclimation per se also affected biliary excretion rate, as 10°C acclimated fish shifted to 14°C had a 125% higher rate (466 nmol·g^{-1}·h^{-1}) than fish acclimated at 18°C and shifted to 14°C (207 nmol·g^{-1}·h^{-1}).

As in our previous study (Curtis et al. 1986), presence of less than 11 nmol [^{14}C]taurocholate equivalent per gram of intestine sampled in the vicinity of the common bile duct indicated that ejection of bile from the gallbladder was not a significant contributor to overall [^{14}C]taurocholate distribution at 1 h after injection (data not shown). There was no significant effect of the thermal treatments on gallbladder bile volume or liver weight, thus removing these factors as possible artifacts.

Hepatic Blood Flow

Liver surface blood flow as measured by LDBF was linearly correlated with total hepatic blood flow in the isolated perfused rat liver (Shepherd et al. 1983). To verify that LDBF could detect changes in overall hepatic perfusion, we occluded the portal vein with mechanical pressure while liver surface blood flow was monitored. As shown in Fig. 2, LDBF almost instantly detected decreased hepatic perfusion. Four replicates of this procedure at four different sites on the liver yielded an average voltage decrease of 51 ± 8%. Therefore, although LDBF only measures surface blood flow, it is also useful as a tool to measure relative total hepatic perfusion in vivo. If we make the reasonable assumption that total hepatic perfusion is linear to LDBF signals in fish as it is in mammals, then fish acclimated to and measured at 18°C had a much greater hepatic blood flow (50–63% increase in voltage) than fish acclimated to and measured at 14 or 10°C (Fig. 3). Acclimation per se did not affect hepatic blood flow, as 10°C acclimated fish shifted to 14°C had the same value as 18°C acclimated fish measured at 14°C.

Mg^{2+}- and Na^+,K^+-ATPase

There was no apparent difference in purity of LPM-1 or LMP-2 from 10 and 18°C acclimated trout. The 5′-nucleotidase activity expressed relative to crude homogenate activity was 4.6 and 30 for LPM-1 and LPM-2, respectively. Glucose-6-phosphatase activity for the same fractions was 0.5 and 1.9, and succinate dehydrogenase activity was 1.5 and 0.6. These data indicated selective enrichment of LPM without significant enrichment of mitochondria or microsomes.

In the initial series of experiments, we characterized some basic properties of Na^+,K^+- and Mg^{2+}-ATPase from rainbow trout LPM: the Mg^{2+} dependence of the Mg^{2+}-ATPase, the more specific Na^+ and K^+ requirement of the Na^+,K^+-ATPase, pH and temperature dependence, and Michaelis–Menten constants. Unless otherwise stated, the preparation used for ATPase assays was LPM-1 and the assays were run at 18 and 37°C with 3 m*M* ATP, 3 m*M* MgCl, and 50 m*M* imidazole, pH 7.4. To verify the Mg^{2+} dependence of Mg^{2+}-ATPase, the assay was run with varying concentrations of $MgCl_2$ with no NaCl or KCl. Activity was negligable (10%) at 0 m*M* $MgCl_2$ and optimal at 3–4 m*M* $MgCl_2$. Furthermore, the addition of the divalent cation chelator EDTA (8 m*M*) to the reaction mixture (3 m*M* $MgCl_2$, 100 m*M* NaCl, and 10 m*M* KCl) abolished more than 90% of ATPase activity, indicating that the Na^+,K^+-ATPase is also Mg^{2+} dependent. Oligomycin (15 μ*M*) reduced Mg^{2+}-ATPase activity by less than 20%, indicating slight mitochondrial ATPase contamination. All subsequent assays were run in the presence of oligomycin. To

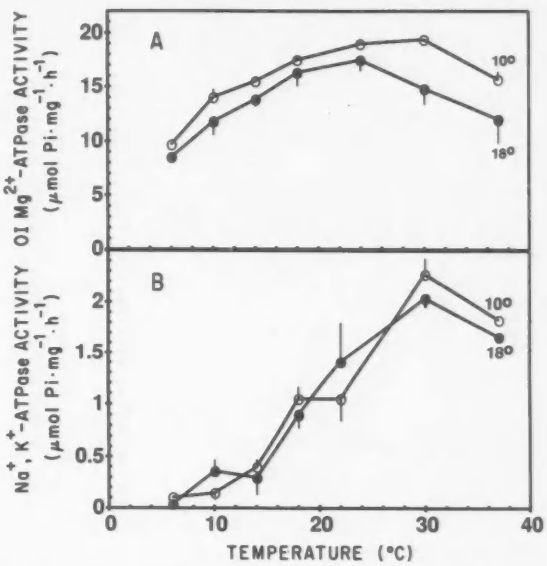

FIG. 4. Effect of temperature on LPM ATPase activities from 10 and 18°C acclimated trout. (A) LPM-2 MG^{2+}-ATPase; (B) LPM-1 Na^+, K^+-ATPase. Each line represents the mean of two separate experiments and each experiment represents material pooled from three to five fish. Vertical lines represent ± 1 SE.

verify that ouabain inhibited Na^+,K^+-ATPase, parallel assays were run using choline chloride as a substitute for NaCl and KCl. The percent inhibition of total ATPase in the presence of 100 m*M* NaCl, 10 m*M* KCl, and 0.5 m*M* ouabain was the same as that with 110 m*M* choline chloride with no NaCl, KCl, or ouabain. The pH optimum of toad skin Na^+,K^+-ATPase increases with decreasing temperature (Park and Hong 1976). We attempted to stimulate the Na^+,K^+-ATPase at low temperatures (10, 14, and 18°C) by varying the pH from 7.2 to 8.0. At 18°C, there was a rather distinct pH optimum of 7.4, but at 14 or 10°C, the Na^+,K^+-ATPase pH optimum was broad, between 7.4 and 7.8. Therefore, by raising the pH, we were unable to significantly stimulate the Na^+,K^+-ATPase at low temperatures.

The temperature/activity curves revealed that Na^+,K^+-ATPase was much more temperature dependent than Mg^{2+}-ATPase. Thermal acclimation did not alter these temperature activity curves in such a way as to explain thermal modulation of biliary excretion (Fig. 4). Kinetic analysis of Mg^{2+}-ATPase and Na^+,K^+-ATPase of LPM-1 from 10 and 18°C acclimated trout was done at 18 and 37°C. The former temperature was selected as a physiologically relevant one at which good activity of both ATPases occurred and the latter one as near optimal activity but likely beyond any lipid phase transition temperature. These data were plotted as a Lineweaver–Burk plot and Michaelis–Menten constants calculated from the line of least squares (Fig. 5). There was little if any effect of thermal acclimation on the K_m or V_{max} for either enzyme assayed at 37°C. However, an assay temperature of 18°C revealed that the K_m for Na^+,K^+-ATPase from 10°C acclimated fish (0.14 m*M*) was ninefold lower than from 18°C acclimated fish (1.11 m*M*).

Temperature/activity profiles for LPM-2 Mg^{2+}-ATPase

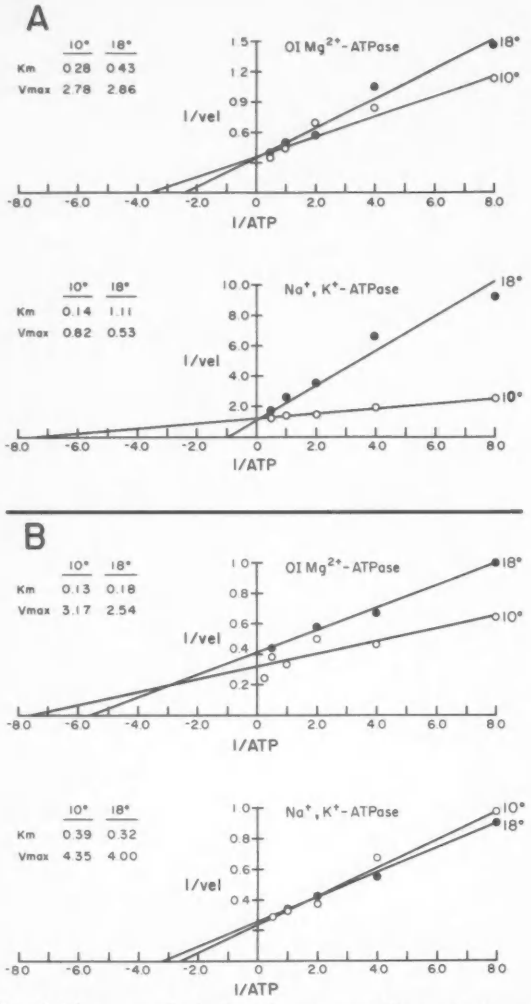

FIG. 5. Lineweaver–Burk plot of measured ATPase activities of LPM-1 from 10 and 18°C acclimated trout mesured at (A) 18°C and (B) 37°C. K_m in mM and V_{max} in $\mu mol \cdot mg^{-1} \cdot h^{-1}$. Each line was calculated by least squares estimation from the mean of three separate experiments. Each experiment represents material pooled from three to four fish.

from 10 and 18°C acclimated fish were determined under conditions shown in Fig. 4 for LPM-1. No differences between temperature acclimation groups were seen (data not shown).

Discussion

Biliary excretion of [^{14}C]taurocholate was affected by both acclimation temperature and acute changes in body temperature. Several results indicated a hepatic rate limiting site for these effects. Earlier work with i.p.-injected [^{14}C]taurocholate demonstrated similar absorption rate constants but a shorter plasma half-life in 18 as compared with 10°C acclimated rainbow trout (Curtis et al. 1986). Also, effects on alternative routes of [^{14}C]taurocholate excretion (i.e. via urine or gills) or on distribution to tissues other than liver or bile were undetected. We therefore dismissed the possibility that thermally modulated hepatobiliary performance was artifactual and focused on mechanisms underlying it.

Hepatic clearance is a measure of the rate at which compounds are removed from the plasma by the liver and is influenced by two independent biological variables: hepatic blood flow and intrinsic clearance (Klaassen and Watkins 1984). For compounds with a high intrinsic clearance, hepatic blood flow is rate limiting and total hepatic clearance varies in direct proportion to blood flow. Taurocholate has a very high intrinsic clearance in dogs, with 90% removed from plasma during a single pass through the liver (O'Maille et al. 1967). That study also demonstrated a linear relationship between taurocholate clearance and hepatic blood flow. Taurocholate is an endogenous bile acid in rainbow trout and when administered at 10 $\mu mol \cdot kg^{-1}$, liver to plasma ratios varied from 10 to 20 (Curtis et al. 1986), supporting the idea that taurocholate has a high intrinsic clearance and that hepatic blood flow could be rate limiting for plasma clearance. Our results also suggest that hepatic blood flow may be rate limiting for biliary excretion of taurocholate but only when fish are held at a constant temperature. Fish shifted up 4° (to 14 and 18°C) had higher rates of biliary excretion than those acclimated and tested at 14 and 18°C, respectively. Fish shifted down 4° (to 14 and 10°C) had lower rates than those acclimated and tested at 14 and 10°C, respectively (Fig. 1). This phenomenon was not observed for hepatic blood flow (Fig. 3). Thus, temperature acclimation per se influenced hepatobiliary performance via another mechanism.

Thermal acclimation alters membrane lipid composition in rainbow trout liver (Hazel 1979), goldfish intestine (Smith 1967), and goldfish synaptosomes (Cossins 1977). This process was termed homeoviscous adaptation and was proposed to involve a shift in membrane lipid composition to maintain a relatively constant membrane fluidity (1/viscosity) in the face of long-term (2–3 wk) changes in environmental temperature (Cossins 1977). Fatty acid composition and fluidity of the lipid bilayer membrane affected the activity of membrane-bound enzymes such as Na^+,K^+-, Ca^{2+}-, and mitochondrial Mg^{2+}-ATPases (Kimelberg 1976; Sandermann 1978). Na^+,K^+-ATPase in particular had quite specific lipid requirements for maximal activity (Kimelberg 1976). Our analysis revealed that rainbow trout LPM Na^+,K^+-ATPase shares many properties with those reported for other tissues and organisms: Na^+,K^+ and Mg^+ dependence, oubain inhibition, K_m, and temperature/activity relationship (Boyer and Reno 1975; Giles and Vanstone 1976; Smith 1967). It is intriguing that the Na^+,K^+-ATPase from both homeotherms and poikilotherms had a dramatic drop in activity in vitro below 20°C. Comparative enzyme analyses usually use specific activity at saturating substrate concentration as a measure of enzyme performance, a condition which may be rare in vivo. A more realistic measure of enzyme performance may be K_m (Somero 1969). At low substrate concentrations, changes in enzyme–substrate affinity may be far more important than changes in V_{max} in governing enzyme activity (Atkinson 1966). Indeed, our studies showed a 5.3-fold decrease in V_{max} but a 2.8-fold increase in enzyme–substrate affinity (as determined by K_m) with decreasing temperature (Fig. 5, 10°C acclimation group). Temperature acclimation per se also affected the K_m of the Na^+, K^+-ATPase: fish acclimated at 10°C had a ninefold decrease in K_m versus 18°C acclimated fish (Fig. 5). Thus acclimation-induced changes in the membrane-bound Na^+,K^+-

ATPase, probably mediated via the lipid microenvironment, were only detectable at physiologically relevant substrate and temperature conditions. Temperature effects on sodium-coupled taurocholate transport not attributable to altered hepatic flow may be due to acclimation-induced changes in the LPM Na^+,-K^+-ATPase or other uncharacterized biochemical changes. LPM Mg^{2+}-ATPase was functional across a wide temperature range (6–37°C, Fig. 4) and was only slightly if at all affected by thermal acclimation (Fig. 4).

Our work on thermally modulated biliary excretion of [^{14}C]taurocholate may explain the work of others who found increased elimination of xenobiotics from tissues of aquatic animals at warmer temperatures which were not readily explained by biotransformation (Varanasi et al. 1981; Niimi and Palazzo 1985; Little et al. 1985). Biliary excretion of some xenobiotics in mammals is thought to occur via the same carrier systems used to transport bile salts, while biliary excretion of other agents may be stimulated indirectly by bile salt transport into bile (Klaassen and Watkins 1984). Experience with biliary excretion of organic anions by rainbow trout indicated that the processes involved are extremely similar to those in mammals (Schmidt and Weber 1973; Curtis 1983; Curtis et al. 1986). We therefore contend that the results reported herein are reflective of aspects of hepatobiliary performance not only related to taurocholate but to biliary excretion in general.

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Vertical Transport of Oxygen into the Hypolimnion of Lakes

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Cornett, R. J., and F. H. Rigler. 1987. Vertical transport of oxygen into the hypolimnion of lakes. Can. J. Fish. Aquat. Sci. 44: 852–858.

Rates of vertical transport of oxygen into the hypolimnion were calculated by multiplying the vertical eddy diffusivity coefficients, determined from the heat budget, by the measured gradients in oxygen concentration. In 12 lakes, transport ranged from 0 to 70 mg $O_2 \cdot m^{-2} \cdot d^{-1}$ and was insensitive to the depth defining the upper boundary of the hypolimnion. Oxygen was transported into the hypolimnion of lakes with a thinner hypolimnion and out of the hypolimnion of lakes with a thick hypolimnion. Transport averaged 4% of the measured total rate of hypolimnetic oxygen depletion and <10% of the depletion rate in individual strata. Pooling these results with published estimates of oxygen gradients and eddy diffusivities suggests that in lakes with different trophic status (phosphorus levels 4–100 $\mu g \cdot L^{-1}$) and size (areas from 0.4 to 70 km^2), vertical oxygen transport accounts for less than 15% of the hypolimnetic oxygen deficit. Oxygen depletion models will gain relatively little precision by including a vertical transport flux parameter.

Les auteurs ont calculé les taux de transport vertical de l'oxygène dans l'hypolimnion en multipliant les coefficients de diffusivité verticale d'un tourbillon, déterminés à partir du bilan thermique, par les gradients quantifiés de la concentration d'oxygène. Dans 12 lacs, le transport a varié de 0 à 70 mg $\cdot m^{-2} \cdot j^{-1}$ de O_2, indépendamment de la profondeur déterminée à partir de la limite supérieure de l'hypolimnion. Dans les lacs où l'hypolimnion était mince, les auteurs ont observé un transport d'oxygène vers l'hypolimnion tandis que dans les lacs où il était épais, l'oxygène était transporté à l'extérieur de celui-ci. Le transport s'élevait en moyenne à 4 % du taux total quantifié d'appauvrissement en oxygène dans l'hypolimnion et à moins de 10 % du taux d'appauvrissement dans les couches individuelles. Le regroupement de ces résultats avec les estimations publiées de gradients d'oxygène et de diffusivité de tourbillon porte à croire que dans les lacs de différents niveaux trophiques (teneur en phosphore de 4 à 100 $\mu g \cdot L^{-1}$) et de différentes superficies (de 0,4 à 70 km^2), moins de 15 % du déficit en oxygène dans l'hypolimnion est dû au transport vertical de l'oxygène. L'inclusion d'un paramètre relatif au transport vertical n'apportera qu'un faible accroissement de la précision des modèles d'appauvrissement en oxygène.

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During the period of thermal stratification, concentrations of dissolved oxygen in the hypolimnion of a lake decrease. The decrease in hypolimnetic oxygen concentration is the net result of processes removing oxygen (sediment oxygen demand, and respiration in the water) and processes adding oxygen (pelagic and benthic photosynthesis and vertical turbulent transport of oxygen). The importance of vertical mixing of oxygen into the hypolimnion is unclear. The sensitivity of simulations of dissolved gas concentrations in stratified lakes to mixing (Bella 1970; Fleming 1974; Imboden 1974; Newbold and Liggett 1974) suggests that vertical transport may be operative. By correcting measures of hypolimnetic oxygen depletion for oxygen transport, Hutchinson (1957), Burns (1976), Mitchell and Burns (1978), Rich and Devol (1978), and Snodgrass and Dalrymple (1985) all implied that transport was an important component of the hypolimnetic oxygen budget. However, Li (1973) concluded that oxygen transport into the hypolimnion of Lake Zurich was not important. Published estimates of transport range from 5 to 250% of the oxygen deficit (Table 1). This suggests that transport may be more important in some classes of lakes than in others (Cornett and Rigler 1979, 1980; Charlton 1980).

In this paper we report estimates of the turbulent transport of oxygen in 12 lakes and test the hypothesis that transport is a negligible contribution to the oxygen mass balance in the hypolimnion. By pooling these measures with published data to produce a simple empirical equation, we have estimated the significance of oxygen transport in a diversity of lakes.

Methods

The study lakes were located in southwestern Quebec, Canada (45°16′N, 72°9′W) (see Cornett and Rigler 1984). Samples for oxygen concentrations were collected over the deepest location on each lake at approximately weekly or biweekly intervals. Five to 15 discrete depths within the hypolimnion were sampled. Sampling depths for oxygen and temperature were uniformly spaced below the epilimnion (1- or 2-m intervals) in shallow lakes (<25 m), while more depths were sampled in the upper zones of thicker hypolimnia, since these strata represent a larger volume of the hypolimnion.

Oxygen was analyzed by the methods of Carpenter (1965) and Bryan et al. (1976) (see Cornett 1982; Cornett and Rigler 1984). Temperature profiles were measured in situ at 1-m intervals to 20 m (or 0.25-m intervals in lakes <20 m deep),

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TABLE 1. Summary of published estimates of vertical transport (VT) and AHOD values. Z is the mean depth of the lake. Central and eastern refer to the two distinct hypolimnetic basins of Lake Erie. Transport of oxygen into the hypolimnion is defined as negative.

Lake	Z (m)	AHOD ($mg \cdot m^{-2} \cdot d^{-1}$)	VT ($mg \cdot m^{-2} \cdot d^{-1}$)	Reference
Durham	2.4	180	− 56	Rich and Devol 1978
Lawrence	5.9	66	−150	Rich and Devol 1978
Mirror	5.6	146	−125	Rich and Devol 1978
Mendota	12.0	570	−217	Hutchinson 1957
Johnson	17.1	546	− 80	Mitchell and Burns 1978
Hayes	18.7	915	−120	Mitchell and Burns 1978
Central	18.3	380	− 46	Burns 1976
Eastern	24.4	613	− 30	Burns 1976
Zurichsee	50.0	1200	40	Li 1973
Ontario	86.0	1300	130	Snodgrass and Dalrymple 1985

2-m intervals from 20 to 30 m, 2.5-m intervals from 30 to 40 m, and 10-m intervals at depths below 40 m. A Fenwall thermistor bead attached to a Data Precision multimeter provided a very stable system capable of resolving resistance changes of 0.001 Ω (about 0.007°C). Resistances were converted to temperatures by calibrating the system against a platinum resistance thermometer and a NBS mercury thermometer. Extinction coefficients were determined from light profiles measured in situ with a GEM photometer at 1-m intervals from 2 to 20 m or until the measured values were below the resolution of the meter. Lake levels were measured on each sampling date and were used to correct the measurements made at slightly different depths (relative to lake bottom) to a constant depth. The lake level corrections (<1 m) changed the estimates of vertical eddy diffusivity by less than 3% in all lakes but one. It was not necessary to correct the oxygen data because the vertical concentration gradients were not as steep as the temperature gradients.

Areal rates of hypolimnetic oxygen depletion (AHOD) were calculated using the methods of Lasenby (1975) and Cornett and Rigler (1980). On each sampling date the mass of oxygen in the hypolimnion was calculated and regressed against time to estimate the AHOD. For the purpose of this calculation and all other calculations described below, the upper boundary of the hypolimnion was defined at one depth for the season based upon summer average temperature profiles (method of Cornett and Rigler 1979; Cornett 1982).

The average summer vertical transport of oxygen across the upper boundary of the hypolimnion (VT, in $mg \cdot m^{-2} \cdot d^{-1}$) was calculated by multiplying the vertical eddy diffusivity coefficient (K_z) by the vertical gradient in oxygen concentration (dO_2/dz, in $mg \cdot m^{-3} \cdot m^{-1}$, hereafter $mg \cdot m^{-4}$) calculated from the derivatives of cubic spline polynomials (Ahlberg et al. 1967) fit to the oxygen concentration versus depth data:

$$(1) \quad VT = K_z \cdot dO_2/dz.$$

The vertical gradient in oxygen concentration used in the calculations is the summer average gradient across the depth delimiting the upper boundary of the hypolimnion. Horizontal variability in all variables was assumed to be small (Smith and Bella 1973; Charlton 1980; Quay et al. 1980; Stauffer 1985).

K_z values were calculated from the change in heat content of the lake below the upper boundary of the hypolimnion (Z_u). Heat content was calculated by numerically integrating the product of the temperature (T_z) and the area (A_z) at depth z. The average seasonal rate of heating over time (t), corrected for inputs of solar radiation (R_z), was determined by regression. Inputs of solar heating were calculated from the measured surface irradiance and extinction coefficients corrected for scattering and solar spectral composition (Bachman and Goldman 1965). Using the flux-gradient method (Powell and Jassby 1974):

$$(2) \quad K_z = \frac{-1}{\frac{dT}{dz}} \cdot \frac{1}{A_z} \cdot \frac{d \int_{Z_{max}}^{Z_u} A_z \cdot T_z \cdot dz}{dt} - R_z - K_{molecular}.$$

The summer average temperature gradient (dT/dz) across the upper boundary of the hypolimnion was calculated by averaging the derivatives of cubic spline polynomials fit to the temperature–depth profiles. Gradients and fluxes are directional, vector quantities and may be positive or negative. We have adopted the usual limnological convention and defined increasing depth below the lake surface as positive. Therefore, dT/dz is negative and K_z is positive in all calculations. Also, dO_2/dz and VT are positive when oxygen is transported out of the hypolimnion and negative when turbulence transports oxygen into the hypolimnion.

Results and Discussion

Comparison of VT and AHOD

Measured total hypolimnetic heating rates ranged from 0.08 to 13 $J \cdot m^{-2} \cdot d^{-1}$ (Table 2). The absolute and relative contributions of solar radiation inputs and turbulent mixing to the heating differed from lake to lake. Solar radiation inputs contributed from <1 to >100% of the measured changes in hypolimnetic heat content. The relative importance of solar radiation was greatest in sheltered lakes with steep metalimnetic temperature gradients. In one lake, Lake Truite, the calculated rate of solar radiation input was significantly greater than the measured heating rate (Table 2). At present we cannot explain this difference. In two other lakes, solar radiation accounted for about 100% of the hypolimnetic heating. In most other lakes the turbulent transport of heat from the metalimnion into the hypolimnion was the dominant heating mechanism.

The average summer K_z values measured in this study ranged over two orders of magnitude (Table 3). All lakes were stratified during the entire summer period ($dT/dz > 1°C \cdot m^{-1}$

TABLE 2. Total rate of hypolimnetic heating (HR), the fraction of heating attributed to solar radiation (S), and the average summer temperature gradient measured across the upper hypolimnetic boundary (dT/dz).

	1979			1980		
Lake	HR ($J \cdot m^{-2} \cdot d^{-1}$)	S (%)	$\frac{dT}{dz}$ ($°C \cdot m^{-1}$)	HR ($J \cdot m^{-2} \cdot d^{-1}$)	S (%)	$\frac{dT}{dz}$ ($°C \cdot m^{-1}$)
Brompton	0.38	6	−1.35	0.96	1	−0.82
Central	0.26	6	−0.27	1.2	1	−0.33
Fitch	0.17	51	−1.30	0.18	6	−0.83
Petite Brompton	—	—	—	0.27	30	−0.86
Magog	0.25	18	−0.98	0.51	27	−0.55
Lovering	0.42	19	−0.80	0.71	7	−1.00
Lyster	0.10	100	−1.24	0.06	100	−1.00
Massawippi	0.75	28	−0.67	1.0	15	−0.79
North	1.3	22	−1.35	0.96	11	−0.47
Orford	0.27	100	−1.18	0.08	99	−0.66
Stukely	0.34	70	−1.40	0.63	13	−1.05
Truite	0.18	176	−1.41	0.08	180	−0.79

TABLE 3. Coefficients of vertical eddy diffusivity (K_z) and gradients in oxygen concentration (dO_2/dz) at the depth of the upper boundary of the hypolimnion (Z_u). All values were calculated from 6 to 15 measures and averaged over the period of stratification. The standard errors (SE) were calculated by propagating errors using the techniques of Beverington (1969). (Central, North, and Fitch are three separate basins of Lake Memphremagog.)

	1979					1980				
Lake	Z_u (m)	K_z ($m^2 \cdot d^{-1}$)	SE	dO_2/dz ($mg \cdot m^{-4}$)	SE	Z_u (m)	K_z ($m^2 \cdot d^{-1}$)	SE	dO_2/dz ($mg \cdot m^{-4}$)	SE
Brompton	12	0.053	0.017	44	72	13	0.264	0.034	− 44	11
Central	20	0.212	0.040	121	31	19	0.866	0.135	66	25
Fitch	12	0.01	0.002	−591	132	12	0.037	0.007	−580	123
Petite Brompton						9	0.043	0.008	−252	90
Magog	12	0.040	0.014	−435	80	10	0.135	0.033	−229	44
Lovering	10	0.094	0.042	−204	74	10	0.170	0.038	−309	55
Lyster	12	0.0	0.035	78	28	12	0.0	0.118	44	12
Massawippi	11	0.188	0.046	79	75	11	0.269	0.065	14	20
North	12	0.169	0.073	−248	70	13	0.422	0.066	−164	29
Orford	11	0.0	0.029	−550	135	12	0.0	0.010	−343	181
Stukely	11	0.01	0.003	82	38	11	0.111	0.027	51	10
Truite	7	0.0	0.012	−795	155	8	0.0	0.023	−355	55

across the thermocline). Higher K_z values measured in 1980 than in 1979 reflect the climatic differences between the two years, the earlier start of the sampling program during 1980 (≈May 15 vs. June 12, 1979), and the slower establishment of strong temperature gradients during 1980. Rates of hypolimnetic heating were higher in 1980 and the vertical gradients in temperature across the upper boundary of the hypolimnion were lower in 1980 (Table 2).

Oxygen concentrations measured in the thermocline were not always higher than those measured within the hypolimnion. To distinguish the direction of the gradient, we adopted the convention of defining dO_2/dz as negative when higher oxygen concentrations were measured above Z_u than below (Table 2). The positive gradients were observed in the deeper, more oligotrophic lakes. Larger negative gradients in oxygen concentrations were measured in the shallower lakes. The different gradients resulted from different rates of respiration in the hypolimnion and thermocline (Hutchinson 1957; Shapiro 1959; Cornett and Rigler 1987).

Although the errors in K_z and dO_2/dz were relatively large, the rates of oxygen transport (VT) were very small (Table 4). In most lakes, oxygen transport was more than one order of magnitude less than the AHOD value. The largest absolute rates of VT, 57 and −69 $mg \cdot m^{-2} \cdot d^{-1}$, were calculated in the central and north basins of Lake Memphremagog. The highest relative rates of vertical transport were 14% of the AHOD in Lake Lovering and 12% of the value measured in the north basin of Lake Memphremagog during 1980. Transport was less than 10% of the AHOD values measured in these lakes during 1979. In all other lakes, VT was less than 10% of the measured AHOD value during both years of study (Table 4). Pooling the data from all lakes, the average rate of vertical transport was 11.6 $mg \cdot m^{-2} \cdot d^{-1}$ in 1979 and 22 $mg \cdot m^{-2} \cdot d^{-1}$ in 1980.

The upper boundary of the hypolimnion was defined operationally from the temperature profile (Cornett 1982; Cornett and Rigler 1980). Since that definition may not be agreed upon by all investigators, we checked the sensitivity of these conclusions to changes in the definition of the depth delimiting the upper boundary of the hypolimnion. Placing the boundary 1 m

TABLE 4. Average summer areal hypolimnetic oxygen deficits (AHOD, mg $O_2 \cdot m^{-2} \cdot d^{-1}$), rate of vertical oxygen transport (VT, mg $O_2 \cdot m^{-2} \cdot d^{-1}$), and error estimates for each variable (SE). VT values labelled with an asterisk are calculated using K_z values that are less than five times greater than the molecular diffusivity of heat and are greater than true values (see text).

Lake	1979				1980			
	AHOD	SE	VT	SE	AHOD	SE	VT	SE
Brompton	326	16.0	2.4*	4.0	389	7.6	−11.5	3.2
Central	586	27.2	25.6	8.2	715	55.7	56.8	23.2
Fitch	354	33.6	−5.9*	1.8	519	26.0	−21.3*	6.0
Petite Brompton					414	28.8	−10.8*	19.0
Magog	523	18.9	−17.3*	6.8	649	22.9	−35.1	10.1
Lovering	293	12.3	−19.1	11.0	367	4.9	−52.6	15.1
Lyster	288	11.5	0.0*	1.0	348	13.0	0.0*	4.2
Massawippi	587	49.6	14.9	14.6	710	22.5	3.8	5.5
North	538	60.0	−42.0	21.7	584	14.3	−69.1	16.2
Orford	304	17.2	0.0*	3.9	408	44.7	0.0*	1.8
Stukely	243	7.1	−0.8*	0.4	309	6.9	5.7	1.8
Truite	207	41.1	0.0*	1.9	208	15.0	0.0*	1.3

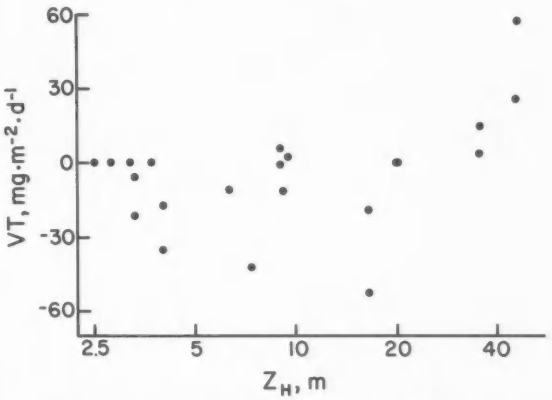

FIG. 1. Vertical transport of oxygen in each lake (VT) plotted against the mean depth of the hypolimnion (Z_H). Note the logarithmic x-axis.

deeper in the water column increased the magnitude of the VT values by an average of 11 mg·m^{-2}·d^{-1} during 1979 and 4 mg·m^{-2}·d^{-1} during 1980. Raising the boundary 1 m above the stated values (Table 3) increased the magnitude of the VT values by an average of 4 mg·m^{-2}·d^{-1} in 1979. In 1980, the same change would decrease the magnitude of the VT values by 4 mg·m^{-2}·d^{-1}. The transport estimates are not sensitive to the definition of the upper boundary of the hypolimnion in these study lakes.

Cornett and Rigler (1980) postulated that the amount of oxygen transported into the hypolimnion of shallow lakes was significantly greater than that into the hypolimnion of deep lakes. This hypothesis was based upon the observation that VT is a larger percentage of AHOD values in shallow lakes (Table 1), a systematic difference between the AHOD values of basins of the same lake with hypolimnia of differing depths (Charlton 1980; Cornett and Rigler 1980), and the observation that larger negative values of dO_2/dz are found in shallow lakes (Table 3; see Fig. 2). We tested for a relationship between VT and hypolimnetic depth using Kendall's nonparametric rank correlation and found a significant positive relationship (Fig. 1, $\tau = 0.53$, $p < 0.01$). The rank correlation between the relative importance of transport (VT/AHOD) and the mean depth of the hypolimnion was also positive and significant ($\tau = 0.48$, $p < 0.01$). However, Cornett and Rigler (1980) postulated that a strong positive correlation between transport and the mean depth of the hypolimnion would explain differences in AHOD values of greater than 100 mg·m^{-2}·d^{-1}. Clearly, the differences in transport between the lakes were not large enough to explain the variations in measured AHOD values shown in Table 3.

Seasonal Variation in Vertical Transport

In the analyses presented above, the average VT values were calculated over the entire summer period. To determine whether VT values differed from the seasonal means during shorter time periods, we calculated VT between each sampling date. In all lakes the measured dO_2/dz values increased and the K_z values decreased during the period of stratification. Measurements from the north basin of Lake Memphremagog are shown as an example (Table 5). In this basin, VT values estimated over these short periods varied by more than one order of magnitude. However, in this basin, VT values were less than 10% of the AHOD in all but one of the time intervals that we examined. Similar seasonal changes in VT were seen in the other lakes, but the seasonal changes were smaller. The average VT value in the north basin calculated from the short-term VT estimates (−30 mg·m^{-2}·d^{-1}, Table 5) was less than the summer average (−55 mg·m^{-2}·d^{-1}, Table 4). This difference was not a mistake. It occurred because K_z is a nonlinear function of the temperature gradient, and the average of a nonlinear function will not equal the function evaluated at the average dT/dz value. Using the average of short-term K_z values would decrease average estimates of VT below those reported in Table 3.

Transport within the Hypolimnion

Although the rate of transport of oxygen into the hypolimnion was low, this does not preclude the possibility that significant amounts of transport occur within the hypolimnion between adjacent strata. We calculated vertical oxygen gradients (Fig. 2) and coefficients of vertical eddy diffusivity (Fig. 3) within the hypolimnion and observed that they may increase in magnitude below the thermocline as the stability of

TABLE 5. Seasonal variation in the vertical transport of oxygen into the hypolimnion in the north basin of Lake Memphremagog during 1980. Symbols are as defined in Tables 2 and 3. dO_2/dz is the average gradient measured during the period. K_z and AHOD were determined over the same period by methods used to determine the seasonal averages.

Period (Julian days)	K_z ($m^2 \cdot d^{-1}$)	dO_2/dz ($mg \cdot m^{-4}$)	VT ($mg \cdot m^{-2} \cdot d^{-1}$)	AHOD ($mg \cdot m^{-2} \cdot d^{-1}$)
133–136	2.11	− 5	−10	774
136–150	4.90	− 16	−81	537
150–164	1.13	− 47	−54	694
164–179	0.13	− 83	−11	690
179–192	0.09	−147	−13	667
192–206	0.05	−173	− 9	499
206–217	0.08	−201	−16	580
217–234	0.17	−257	−44	451

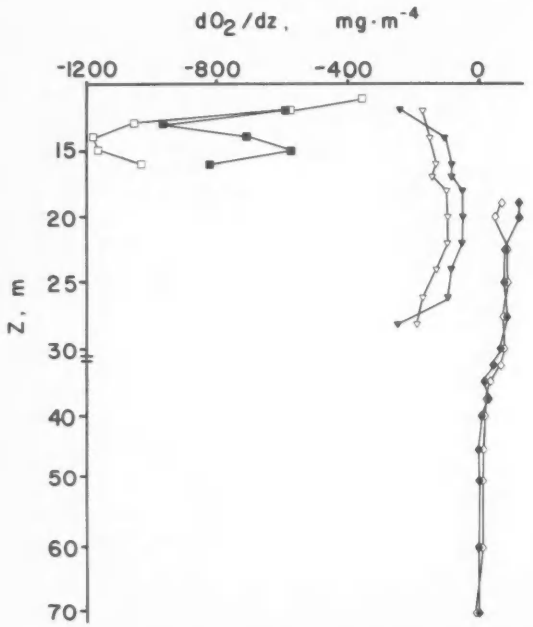

FIG. 2. Vertical profile of gradients in oxygen concentration (dO_2/dz) measured in the three separate hypolimnetic basins of Lake Memphremagog (Fitch Bay 1979 (■) and 1980 (□); north basin 1979 (▼) and 1980 (▽); and central basin 1979 (◆) and 1980 (◇)). Positive gradients were calculated when the concentrations of oxygen measured near the upper boundary of the hypolimnion were lower than the concentrations measured deeper in the water column.

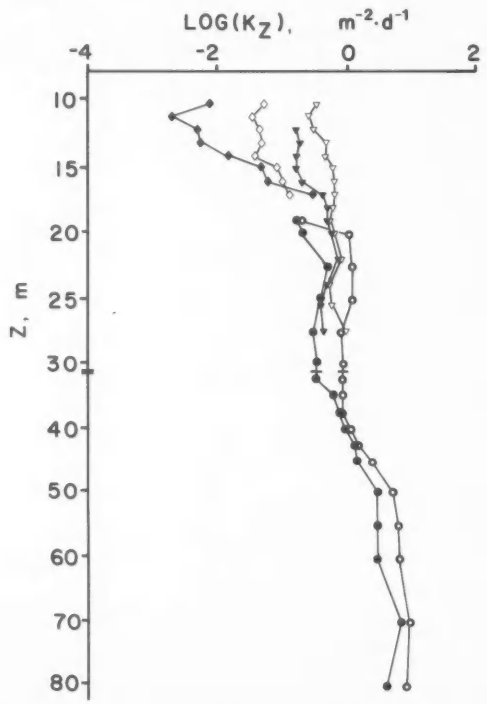

FIG. 3. Vertical profile of coefficients of vertical eddy diffusivity (K_z) measured within the hypolimnion of the north basin (1979 (▼) and 1980 (▽)), central basin (1979 (●) and 1980 (○)), and Fitch Bay (1979 (◆) and 1980 (◇)) of Lake Memphremagog. Note the logarithmic scale of the x-axis and the change in scale of the y-axis.

the water column decreases (Birge and Juday 1911; Quay et al. 1980). Methods used to calculate AHOD, dO_2/dz, K_z, and VT were identical to those used to analyze oxygen depletion and transport within the entire hypolimnion. The values of each variable were calculated for each depth stratum in each lake.

Oxygen was turbulently mixed through the planar surfaces that delineated the boundaries of the different hypolimnetic strata (Fig. 4). However, the flux was continuous and oxygen was transported both into and out of each adjacent stratum. Therefore, the flux into a stratum and the flux out were strongly correlated ($p < 0.001$) and the *net* flux of oxygen (in or out) was much smaller than the *total* flux (Fig. 4). Of the 160 strata studied in 12 lakes, the net amount of oxygen mixed into these strata was greater than 20% of the observed rate of oxygen depletion in only 26% of the strata. The relative importance of transport was much higher in the deepest strata of the hypolimnion (Fig. 4). This occurred because K_z values were usually larger than those measured nearer the thermocline (Fig. 3). In these deep zones, the observed rate of oxygen depletion will underestimate actual rates of depletion unless a correction for transport is made. In the upper layers of the hypolimnion, transport was usually estimated to be less than the uncertainty in the estimated rate of oxygen depletion.

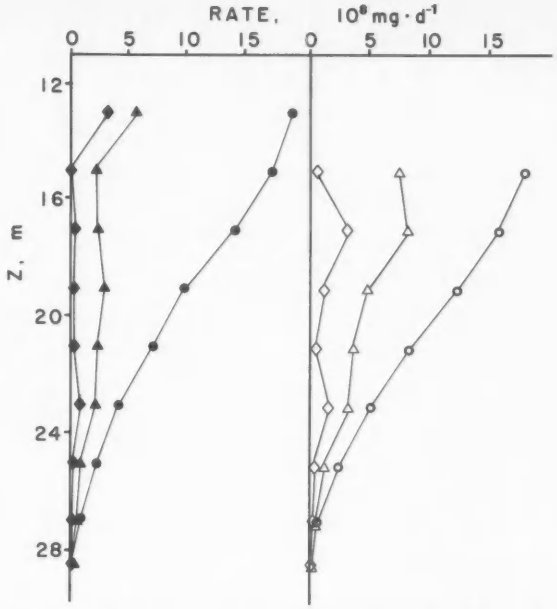

FIG. 4. Seasonal average rates of oxygen consumption within each stratum (1979 (●) and 1980 (○)), total oxygen transport into each stratum (1979 (▲) and 1980 (△)), and the net mass of oxygen added to the strata (1979 (◆) and 1980 (◇)) in the hypolimnion of the north basin of Lake Memphremagog. The variables are shown in the same units to facilitate comparison.

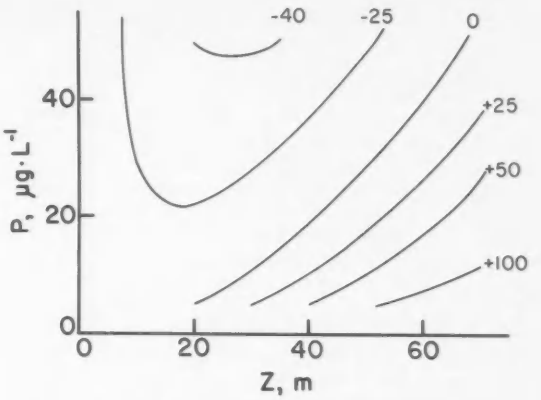

FIG. 5. Isopleths showing estimates of oxygen vertical transport (mg $O_2 \cdot m^{-2} \cdot d^{-1}$) calculated from equations 3 and 4 plotted for different combinations of phosphorus concentration (P) and mean lake depth (Z).

Comparison with Other VT Values

Several lines of reasoning suggest that the transport of oxygen is less than 10% of the AHOD in other lakes. Since K_z values are higher in larger lakes (Mortimer 1942; Snodgrass and O'Melia 1975; Quay et al. 1980), it is significant that even in Lake Memphremagog with a fetch of greater than 40 km, transport was no higher than 15% of the AHOD. However, it could be argued that this resulted from the small oxygen gradients in this oligotrophic lake. To determine the influence of trophy, we estimated the average vertical gradients in oxygen concentration at the upper boundary of the hypolimnion in 11 more eutrophic lakes using published data (available in Cornett 1982). Pooling those data with the data presented in Table 3, the best regression that estimated the vertical oxygen gradients in lakes of different morphometry and different trophy (estimated by the surface water phosphorus concentration (P), in $mg \cdot m^{-3}$) was ($N = 36$, $R^2 = 0.67$, $S_b = 145$):

$$(3) \quad dO_2/dz = -474 - 206 \cdot \log(P) + 387 \cdot \log(Z_{max} - Z_u).$$

Equation 3 predicts that oxygen gradients change *logarithmically* with P and depth and gradients will be largest in shallow, eutrophic lakes. Such lakes will rarely have high K_z values (Snodgrass and O'Melia 1975; Quay et al. 1980). The best correlate of K_z is mean depth. Snodgrass and O'Melia (1975) calculated the best linear regression relating K_z ($m^2 \cdot d^{-1}$) and mean depth (z, in metres) to be

$$(4) \quad K_z = 0.00682 z^{1.18}.$$

Multiplying equations 3 and 4 together and estimating z as $(Z_{max} - Z_u)/2$ provides estimates of VT for lakes with a diversity of morphometric and trophic properties. Values of VT range from -42 to 168 mg $O_2 \cdot m^{-2} \cdot d^{-1}$ over the range of morphometric ($5 \text{ m} < z < 70$ m) and trophic ($5\ \mu g \cdot L^{-1} < P < 50\ \mu g \cdot L^{-1}$) values (Fig. 5). Therefore, VT will be a small fraction of the AHOD in most lakes, since values of AHOD range from 250 to 1000 $mg \cdot m^{-2} \cdot d^{-1}$ in most lakes (Cornett and Rigler 1980).

Our observation that transport is less than 15% of the AHOD agrees with estimates of transport in Lake Erie (Burns 1976), Lake Zurich (Li 1973), Lakes Hayes and Johnson (Mitchell and Burns 1978), and Lake Ontario (Snodgrass and Dalrymple 1985). VT values in Lake Mendota (Hutchinson 1957) and the three small lakes studied by Rich and Devol (1978) appear to be extremely high (Table 1). The high VT values calculated by Rich and Devol resulted from large K_z values (P. H. Rich, Biological Sciences, University of Connecticut, Storrs, CT, pers. comm.). K_z values in these small lakes were much higher than expected (Mortimer 1942; Snodgrass and O'Melia 1975; Quay et al. 1980). High K_z values may be a result of bias introduced by the difficulty in attempting to correct the rate of hypolimnetic heating for the input of solar radiation in very shallow lakes (Rich 1979; see also Table 2).

The apparent difference between Lake Mendota and the lakes investigated in this study occurred because Hutchinson (1957) calculated AHOD and VT values over the time period when a large volume of the hypolimnion was anoxic. Using the AHOD value (Stewart 1975) and gradients in oxygen concentration (data of Birge and Juday 1911) calculated during the period when the hypolimnion was oxic and the K_z value calculated by Powell and Jassby (1974), we calculate VT to be 97 $mg \cdot m^{-2} \cdot d^{-1}$ and the AHOD as 783 $mg \cdot m^{-2} \cdot d^{-1}$ in Lake Mendota. Transport was 14% of the AHOD and the results for Lake Mendota were very similar to other lakes.

Oxygen and Heat Transport

To date, all analyses of the vertical transport of oxygen have used K_z coefficients, derived from the heat budget of the

hypolimnion, to calculate the vertical diffusivity of mass (oxygen). This assumes that heat and mass were transported at the same rate within the water column. In highly stratified regions, however, the turbulent transfer of mass may be more than one order of magnitude less than K_z values calculated from the heat budget (Turner 1968; Hesslein and Quay 1973; Quay et al. 1980). Therefore, the VT values calculated must often be considered upper limits to the correct rate of oxygen transport. In this study, K_z was less than five times greater than molecular diffusion in five and seven lakes in 1980 and 1979, respectively (Table 3). In these lakes, our estimates of oxygen transport are too high. In other lakes with larger K_z values, estimates of oxygen transport will not be biased (Quay et al. 1980), since heat and mass are transported at the same rates. In these lakes, transport of oxygen averaged 5% of the observed rate of oxygen depletion. Estimates of VT were always smaller than the uncertainty in the measured AHOD (Table 4). Models of oxygen depletion will gain relatively little precision if they focus on vertical transport of oxygen across the upper boundary of the hypolimnion. We plan additional studies to extend this conclusion to the vertical transport measurements of other dissolved chemical species. A general model of vertical transport would simplify calculations of the mass balance of all substances.

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Methods of Time Trends Analysis Applied to Contaminant Levels in Canadian Atlantic Cod (*Gadus morhua*)

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Misra, R. K., and J. F. Uthe. 1987. Methods of time trends analysis applied to contaminant levels in Canadian Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 44: 859–865.

Similarities and differences between multiple linear regression (MLR) and analysis of variance (ANOVA) approaches which have been used as two seemingly different methodologies in time trend studies are explained. Difficulties encountered in choosing an appropriate MLR model are presented, and need for identifying outlying observations in a MLR analysis is emphasized. Univariate analyses such as MLR and ANOVA are of restricted scope and may even be misleading when employed on data sets containing several contaminants. Multivariate structures of these data sets have distinct advantages over a univariate model. The use of multivariate analysis of covariance (MANCOVA) for time trends investigation is illustrated with Canadian Atlantic cod (*Gadus morhua*) data. Significant linear time trends are shown, although these did not explain all variations between years.

Les auteurs expliquent les ressemblances et les différences entre une régression linéaire multiple (RLM) et l'analyse de la variance (ANOVA) qui sont utilisées apparemment comme deux différentes méthodes d'étude des tendances temporelles. Les problèmes rencontrés dans le choix d'un modèle approprié de RLM sont présentés; les auteurs soulignent l'importance de relever des observations isolées dans une analyse de RLM. Les analyses à une seule variable comme la RLM et l'ANOVA sont de portée restreinte et peuvent même induire un erreur quand on les utilise avec des séries de données contenant plusieurs erreurs. Les structures à plusieurs variables de ces séries de données ont des avantages marqués sur le modèle à une seule variable. L'utilisation d'une analyse à plusieurs variables de la covariance (MANCOVA) pour les études des tendances temporelles est illustrée à l'aide de données sur la morue de l'Atlantique (*Gadus morhua*) canadienne. Des tendances temporelles linéaires importantes sont révélées quoiqu'elles n'expliquent pas toutes les variations entre les années.

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Since 1967 the International Council for the Exploration of the Sea (ICES) has been interested in the assessment of pollution of marine waters. From their first study of the North Sea and its adjacent waters (ICES 1974), the studies have grown to include the North Atlantic where the member nations of ICES have fishing and other interests (ICES 1977). In addition to providing continuous assurance of the quality of marine foodstuffs with respect to human health and providing an indication of the health of the marine environment, these studies are also aimed at providing an analysis of time trends in contaminant levels in selected fish stocks. The single most important question regarding chemical contamination levels in fish populations is "Are contaminant levels in stocks increasing, staying constant or decreasing over time?" (Scott et al. 1983). The programme and suggested sampling methodologies for studying the three objectives are described in ICES (1984).

In 1977, we began an assessment of a number of contaminants in Atlantic cod (*Gadus morhua*) from the southern part of the Gulf of St. Lawrence using a sampling strategy based on fish samples spread over a wide range of length (Scott et al. 1978). Scott et al. (1981, 1983) gave a useful lead into time trend analyses with their models of multiple linear regression (MLR) of each chemical contaminant on a suite of common size and biological variables. Time trend analysis in the framework of analysis of variance (ANOVA), rather than by the MLR procedure of Scott et al. (1981, 1983), has also been considered (e.g. see Jensen and Lassen 1981; Hansen 1982; Lassen 1983).

There has been much discussion among statisticians as to what are the appropriate statistical methodologies to employ in analyzing data sets of this type for time trends (Uthe et al. 1984). In this paper we state briefly the (1) similarities and differences between the MLR and ANOVA approaches that have been used for time trend analyses, (2) difficulties in choosing an appropriate MLR model and the need to identify outlying observations in trend analysis beyond the level of scatter plots and residuals, and (3) usefulness of multivariate analysis of covariance (MANCOVA) for time trends, and we assess Canadian Atlantic cod data for time trends by MANCOVA.

Materials and Methods

Cod Sampling

Atlantic cod in the Maritime area of Canada are thought to be represented by four major stock divisions that intermix to greater or lesser degrees (Kohler 1968; Hare 1977). Of the four groups, the one present in the southern Gulf of St. Lawrence is believed to be the most discrete, residing south of the Laurentian Channel in the summer and migrating annually eastward to

TABLE 1. Mean concentrations (mg/kg wet wt ± SD) in liver and muscle of 6-yr-old Atlantic cod from NAFO areas 4T and 4Vn (N = 10 for metals, N = 5 for others, *N = 6).

NAFO area	Hg	Pb	As	Cd	Cu	Zn	PCB	Dieldrin	p,p'-DDE	p,p'-DDT	% fat
					Liver						
4T	0.06±0.01	0.71±0.24	2.8±2.4	0.050±0.018	2.1±1.5	5.13±0.16	2.9±1.2	0.073±0.034	0.70±0.35	0.59±0.27	63.0± 6.0
4Vn	0.09±0.02	0.17±0.10	4.8±4.4	0.053±0.037	1.6±2.3	3.2±0.5	1.9±0.4	0.064±0.021	0.57±0.20	0.44±0.15	66.0±11.0
					Muscle						
4T	0.05±0.02	0.21±0.21	1.7±1.6	0.13±0.06	3.1±1.1	10.0±3.8	0.015±0.004	0.001±0.001	0.002±0.001	0.003±0.002	0.21±0.16
4Vn	0.04±0.01	0.28±0.21	2.7±1.5	0.28±0.10	5.1±1.8	13.0±2.9	0.009±0.002	0.002±0.002	0.001±0.001	0.004±0.002	0.41±0.26

overwinter. It is not known if all fish undertake this annual migration or if there is a subpopulation which resides in the Gulf all year. In order to determine if there were significant differences in contaminant levels in cod from the southern part of the Gulf of St. Lawrence (NAFO area 4T) and the overwintering eastward area (NAFO area 4Vn), ten 6-yr-old cod were captured in the summer of 1975 from each area and liver and muscle were analyzed (Table 1). Some statistically significant differences were found (e.g. for Pb), but overall the results suggest that the contaminant histories of the fish from the two areas are similar.

Annual samples were obtained from a single 0.5-h otter board trawl haul within NAFO area 4T (lat. 47°50′06″; long. 63°40′) in the third week of September. In 1979, interlevel variability was assessed by Scott et al. (1981). Two 0.5-h hauls were made in the same spot and separately subsampled. Differences between samples were not significant.

Ten strata based on total length were filled with the first five fish from the haul which fitted the stratum (each stratum was 5 cm long, the overall length range was 15–65 cm). All strata could not always be completely filled. The number of fish taken each year is provided later in the text. The round fish were rapidly frozen aboard ship and kept frozen until dissection. Liver and whole skinned fillets were removed from each fish without allowing the fish to thaw. Whole livers were weighed and homogenized in a washed glass ointment jar using a Polytron homogenizer (Brinkman Instruments). The jar was covered with a washed foil-lined cap and frozen at −40°C. Fillets were weighed and homogenized in a glass Waring Blendor fitted with Teflon gaskets with an equal weight of glass-distilled water, or later processed without additional water in a Hobart Silent Cutter. A portion of this homogenate was stored in the same manner as the liver. All dissection procedures were carried out with a steel knife. Glass-distilled water was processed the same as tissues to yield a "blank" sample for analytical quality control. Samples were rehomogenized (Polytron) prior to subsampling for contaminant measurements.

Trace metal determinations in tissues were carried out following acid dissolution using atomic absorption spectroscopy (Perkin-Elmer model 403 spectrophotometers fitted with deuterium arc background correctors). Graphite tube furnaces (PE HGA 70 and 72) were employed for samples containing low metal concentrations. The method has been described in detail previously for Hg, Pd, Cd, Zn, and Cu (Topping and Holden 1978). Arsenic and Se were determined by the methods of Freeman et al. (1976) and Shum et al. (1977), respectively. Organochlorines were analyzed by the method of Reynolds and Copper (1975) using Aroclor 1254 as a standard for polychlorinated biphenyl determinations. All contaminant concentrations were reported on a wet weight basis. Tissue burdens are simply the concentration of the contaminant (micrograms per gram) multiplied by the weight of the whole tissue in (grams). This paper considers only the results for 1977, 1978, and 1979. The basic data for statistical analysis were reported by Scott et al. (1981, 1983). Our study is based on the analyses of 34, 45, and 45 fish sampled in 1977, 1978, and 1979, respectively. On each individual, the following 19 measurements were taken: (1) concentrations of 13 contaminant–tissue combinations, As–M, Cu–M, Hg–M, Zn–M, As–L, Cd–L, Cu–L, Hg–L, Se–L, Zn–L, PCB–L, α-HCH–L, and HCB–L where M is muscle and L is liver, and (2) six biological characters, age (years), length (centimetres), net weight

(total weight minus liver weight in grams), muscle fat (proportion), liver weight (grams), and liver fat (proportion).

Statistical Considerations

For predicting changes in levels of chemical concentrations in Atlantic cod with time, Scott et al. (1983) developed noninteractive and interactive versions of the MLR, designated as models (1) and (2). In each model, the dependent (response) variable, Y, was $\log_{10}$ (contaminant concentration). Scott et al. (1983) also extended the use of the MLR to their organ (or tissue) burden models (3) and (4). In these burden models, Y was $\log_{10}(W \times$ contaminant concentration) where W was net weight or liver weight. Their noninteractive (rectilinear) model may be written as

$$(1) \quad Y_i = \sum_{p=0}^{7} \beta_p X_{ip} + e_i$$

where $Y_i = \log_{10}$ (contaminant concentration), X_{i0} is a dummy variable which is always unity, X_{i1} is a dummy variable with levels -1, 0, and 1 for 1977, 1978, and 1979, respectively, X_{i2} = age, $X_{i3} = \log_{10}$ (length), $X_{i4} = \log_{10}$ (net weight), $X_{i5} = \log_{10}$ (muscle fat), $X_{i6} = \log_{10}$ (liver weight), $X_{i7} = \log_{10}$ (liver fat), and e_i are independent $N(0, \sigma^2)$, $i = 1,\ldots,n$ (n = total number of fish samples over the 3-yr period). Rationale for employing log-transformed data, indicated above, was provided by Scott et al. (1981, 1983).

Scott et al. (1983) employed the linear regression approach to analyze for time trends by using a linearly coded "regression" variable for year. In the ANOVA approach (Jensen and Lassen 1981; Hansen 1982; Lassen 1983), year was employed as a "class" variable. It is, therefore, important to understand the similarities and differences between the two approaches. Following Li (1964, section 17.6), Bliss (1970, section 18.3), and Draper and Smith (1981, section 2.12), these are outlined as follows. Denoting the coefficient of linear regression of the yearly means Y_j, $j = 1, 2, 3$ on X_1 as β, the hypothesis $\beta = 0$ of the F-test of the linear regression is the same as that of ANOVA. However, the alternative hypotheses in the two approaches are different. This difference affects the power of the F-test. The two degrees of freedom (df) between years could be partitioned as follows:

Source	df
Linear among years	1
Departure from linear (lack of fit)	1
Error (pure error)	$n - 3$

The ANOVA approach pools the first two rows and tests the hypothesis of no year effect against the pure error term. The "regression" approach pools the last two rows as error and tests the hypothesis of no linear trend against this estimate of error.

Outlying observations

A fitted MLR can be distorted considerably by an outlying observation especially when a data set contains a small number of observations, thereby severely restricting its usefulness. Therefore, possible outlying observations must be identified. The following are noted (Draper and Smith 1981; Neter et al. 1983).

(1) Only the Y value of an observation, or its X value(s), or both may be outlying.

(2) For a MLR model which employs two or more independent variables, identification of outlying observations by simple measures such as scatter and residual plots alone are insufficient and more powerful methods for identifying outliers with respect to their X and Y values are needed. For example, from the "hat matrix" method for identifying outlying X observations presented later in the text presentation, it is obvious that, when the model is appropriate, observations with "high leverage" (indicating thereby that their X values are outlying) tend to have smaller residuals. Therefore, detection of outliers from residuals alone may not be sufficient. In small samples, residuals may be correlated to a degree and may not estimate errors adequately.

(3) An observation may outlie with respect to its Y value and/or its X value(s), and yet it may not have an influence strong enough to distort the fitted MLR model. Therefore, overall impact of an observation in the fitted MLR must also be examined.

(4) Finally, an outlier identified statistically need not be rejected automatically. It may still be real data, in which case an understanding and/or an explanation of circumstances which generated it may be required.

Procedures for identifying outliers employed in this presentation were taken from Draper and Smith (1981) and Neter et al. (1983) and are as follows.

(i) Identifying outlying X observations. This was done by using the "hat matrix" (Neter et al. 1983, section 11.5). The ith observation was considered to be outlying with respect to its X values if its "leverage" h_{ii} was large, i.e. greater than twice the mean leverage value, $2p/n$, where p is the number of regression parameters (including the intercept term) in the MLR fitted to n observations.

(ii) Identifying outlying Y observations. This was done by using Studentized deleted residuals which follow the t distribution (Neter et al. 1983, section 11.5). Observation Y_i is identified as an outlier by the (significantly) large value of its t.

(iii) Evaluating the impact of observations on the fitted MLR. The overall measure of the impact of the ith observation on a fitted MLR model was examined by Cook's statistic. Cook's distance measure, D_i (Draper and Smith 1981, section 3.12; Neter et al. 1983, section 11.6), is compared with $F_{p,n-p,1-\alpha}$ to ascertain the percentile value for selected α and will identify an observation which affects the coefficients of a fitted MLR. If the percentile value is ≥ 50, the distance between $\boldsymbol{b}$ and $\boldsymbol{b}_{(i)}$ is considered to be distinctly large, leading to the conclusion that the ith observation has a substantial effect on the coefficients of the MLR. If the percentile is low, e.g. ≤ 20, the ith observation has little influence on the fitted MLR. Intermediate values (i.e. 20–50%) are in the "gray" zone. Observations with these intermediate values would influence the MLR but not to the extent that remedial measures are warranted.

A fitted MLR can be severely distorted by the inclusion of outlier(s). On the other hand, an outlier may have occurred due to the failure of the MLR model. To achieve high reliability of a MLR model, we must include all X variables. This may lead to a large suite of independent variables when we consider including possible extensions to functions of these variables (Draper and Smith 1981, Chap. 6), such as a full second- (or higher) order model. The more independent variables included in a MLR equation, the more useful it should become for predictive purposes. This route of exploring data for an appro-

priate MLR model will, however, not be feasible, as monitoring a large number of variables is not practical and will present additional difficulties or magnify existing ones, such as multicollinearity in the predictor variables, occurrence of spurious effects caused by random sampling variability, obtaining more than one "best" model, detracting from the model's descriptive abilities while adding little to its predictive power, and magnifying the problem of round-off errors (particularly when ill-conditioning occurs in $X'X$). For reasons such as these, we would want to retain as few independent variables in a MLR model as possible. Obviously, then, we need to find a compromise between these extremes. We may do so by following one or both of the following routes.

(1) Totally depend on statistical techniques to select the "best MLR". Several techniques are available (Draper and Smith 1981, Chap. 6). We note (Draper and Smith 1981), however, that (a) no technique works well in all circumstances, or is always better than all the others, or is without any constraint and (b) screening of variables should never be left to the sole discretion of any statistical procedure.

(2) The other route may, therefore, be to take a pragmatic approach, such as starting with the inclusion of only those variables in the MLR which are "key" to the objective of the survey.

We took this approach. In the cod survey, considering that the objective of this study was to investigate time trends, X_{i1}, X^2_{i1} (designated as X_{i8} in the text to follow), and interaction variables generated from their cross products with biological variables X_{ik}, $k = 2,\ldots,7$, were considered as pertinent. Outliers were identified for MLR models which employed independent variables as indicated in the following: (i) model (1) of Scott et al. (1983), variables X_{ik}, $k = 1,\ldots,7$; (ii) model (2) of Scott et al. (1983) which used interaction variables $X_{i1}X_{ik}$, $k = 2,\ldots,7$, and all independent variables of model (1); (iii) model (3), variable X_{i8} and all independent variables of model (1); (iv) model (4), variables X_{i8} and all independent variables of model (2); (v) model (5), X_{i1}, X_{i8}, biological variables X_{ik}, and interaction variables $X_{i1}X_{ik}$ and $X_{i8}X_{ik}$, $k = 2,\ldots,7$; (vi) model (6). We also examined each year's data separately for possible outlier(s) for the MLR which employed only the biological variables, i.e. X_{ik}, $k = 2,\ldots,7$. Burden data were also analyzed for possible outlier(s), using these six forms of the MLR model.

Multivariate analysis

Any MLR or ANOVA is only a "univariate" analysis and included in the multivariate analysis of covariance (MANCOVA), a multivariate model which employs multiple dependent variables and multiple independent variables. Detailed information on multivariate analyses may be obtained from Bliss (1970), Games (1971), Kshirsagar (1972), Harris (1975), Morrison (1976), Gnanadesikan (1977), Mardia et al. (1979), Pimentel (1979), and Srivastava and Carter (1983). The scope of multivariate analyses is given briefly as follows.

(1) Analyses for time trends done in the past have examined chemical contaminants only one at a time. For example, Scott et al. (1983) regressed only one Y variable on X variables in their MLR models. Y variables for 13 contaminant–tissue combinations (in the log scale) in cod data are mutually correlated. "A series of univariate statistical analyses carried out separately for each of the variables is, in general, not adequate as it ignores the correlations among the variables" (Kshirsagar 1972).

(2) Even when time trends can be identified with a single variable, a combined criterion of two or more will make it more effective.

(3) Multivariate analyses can yield additional and/or more meaningful information than univariate analyses pertaining to relationships, interdependence, and relative importance of the characteristics involved. For example, (a) we can examine statistically time trends for differences among contaminants, in addition to those for contaminants, (b) we can establish statistically whether changes in levels of a contaminant with time are due to the contaminant itself or to its correlation(s) with other contaminant(s), and so on.

(4) However, multivariate analyses are not without difficulties. Multivariate significance tests, like their univariate counterparts, are based on normality assumptions. This assumption is stronger at the multivariate level than at the univariate level. It is well known that the common univariate tests are generally quite robust to departures from normality. "Less is known about the robustness of the corresponding multivariate techniques, but the evidence so far suggests similar degree of robustness" (Harris 1975). "...the sampling distributions of many multivariate statistics are approximately normal, regardless of the form of the parent population.." (Johnson and Wichern 1982). Loss in (statistical) power of the multivariate tests of hypotheses in comparison with those based on separate responses may also occur in some situations (e.g. see Roy et al. 1971; Morrison 1976). If an outlier is present in one variable, the entire record must be deleted. Alternative procedures for conducting specific comparisons following the rejection of the overall test for differences among group (year) means exist (e.g. see Harris 1975, Chap. 4). In spite of these difficulties, multivariate analysis is preferred to univariate analysis (a) for reasons given earlier in the text and (b) as indicated in the following quote: "...researchers in all of the sciences — behavioural, biological, or physical — have long since abandoned sole reliance on the classic univariate design for very excellent reasons" (Harris 1975). Univariate analysis may even be misleading sometimes (Kshirsagar 1972).

Let n = total number of fish samples over the 3-yr period, m = number of years + 1 (=4 here), u = number of contaminant tissue combinations (=13 here), and q = number of independent variables. The general form of the MANCOVA model may then be given as (Morrison 1976, section 5.4)

$$\boldsymbol{Y} = \boldsymbol{A\theta} + \boldsymbol{XB} + \boldsymbol{e}$$

where $\boldsymbol{Y}$ is the $(n \times u)$ matrix of observations. $\boldsymbol{A}$ is the $(n \times m)$ design matrix of rank $K(=m-1)$, $\boldsymbol{\theta}$ is the $(m \times u)$ matrix of regression coefficients of the design matrix, $\boldsymbol{X}$ is the $(n \times q)$ matrix of concomitant observations, $\boldsymbol{B}$ is $(q \times u)$ matrix of regression parameters, and $\boldsymbol{e}$ is the $(n \times u)$ error matrix whose rows are independently distributed as $N_u(0, \Sigma)$. $\boldsymbol{A}$ contains the $n \times K$ basis and is linearly independent of $\boldsymbol{X}$. Contaminant–tissue mean vectors of the three years, adjusted for different values of the independent variables, may be compared by MANCOVA. If these adjusted mean vectors differ (as they did in our analysis of cod data, presented later in the text), this would imply that significant linear combinations of contaminant–tissue combinations and/or year groups exist. Identifying all these linear combinations is a difficult task. Considering that the objective of the study was to investigate time trends, we examined the contrasts for linear (time) trend

TABLE 2. Mean, minimum, and maximum values for X and Y variables of (a) concentration data and (b) burden data. All variables except X_2 (age) are transformed to common logarithms.

Variable	1977 (N = 33)			1978 (N = 44)			1979 (N = 44)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
				(a) Concentration data					
X_2 age (yr)	4.0	2	7	4.6	3	7	5.7	3	14
X_3 length (cm)	1.6170	1.4472	1.7924	1.6873	1.5051	1.8261	1.6813	1.4928	1.8261
X_4 net wt. (g)	2.8049	2.2310	3.3134	3.0094	2.4346	3.4553	2.9602	2.4273	3.4216
X_5 muscle fat (%)	−2.2405	−2.3565	−2.1079	−2.1867	−2.4437	−1.9245	−2.2540	−2.5229	−1.9914
X_6 liver wt. (g)	1.3553	0.8808	1.9643	1.6209	0.8921	2.0792	1.5418	0.9345	2.0265
X_7 liver fat (%)	−0.3661	−0.5817	−0.2321	−0.3078	−0.8697	−0.1158	−0.3366	−0.9066	−0.1618
Y_1 As−M (mg/kg)	0.3603	−0.1549	0.7745	0.2414	−0.2518	0.5441	0.2995	−0.2441	0.8414
Y_2 Cu−M (μg/kg)	2.7284	2.6021	2.9542	2.6473	2.3802	3.2112	2.5413	2.2304	3.0334
Y_3 Hg−M (μg/kg)	1.5829	1.1461	1.9731	1.7819	1.3979	2.0682	1.7263	1.4150	2.1239
Y_4 Zn−M (μg/kg)	3.6242	3.5263	3.7459	3.6149	3.5465	3.7396	3.6648	3.5551	3.8915
Y_5 As−L (mg/kg)	0.2908	0.0755	0.5988	0.2427	−0.0044	0.6503	0.3691	0.0969	0.8579
Y_6 Cd−L (μg/kg)	1.7815	1.3424	2.4232	2.1794	1.7634	2.7459	1.8444	1.4150	2.4116
Y_7 Cu−L (mg/kg)	0.5988	0.2553	1.1038	0.6194	0.0569	0.9777	0.8237	0.4829	1.4378
Y_8 Hg−L (μg/kg)	1.3547	0.9031	1.6902	1.4262	1.0000	2.0000	1.3120	1.0000	1.8388
Y_9 Se−L (μg/kg)	1.9134	2.7634	3.1173	3.0219	2.6990	3.3054	3.1307	2.7243	3.5966
Y_{10} Zn−L (mg/kg)	1.1841	1.0128	1.3655	1.1818	0.8976	1.3874	1.2643	1.0141	1.5111
Y_{11} PCB−L (mg/kg)	0.2767	0.0043	0.7193	0.4626	0.1239	0.8075	0.4169	0.1430	0.8716
Y_{12} α−HCH−L (μg/kg)	1.8629	1.4914	2.0755	1.8146	1.3424	1.9868	1.8127	1.2553	2.0253
Y_{13} HCB−L (μg/kg)	1.6279	1.1761	2.0828	1.6582	0.8451	1.9031	1.6247	1.1139	1.8261
				(b) Burden data					
X_2 age (yr)	4.0	2	7	4.6	3	7	5.7	3	14
X_3 length (cm)	1.6170	1.4472	1.7924	1.6873	1.5051	1.8261	1.6813	1.4928	1.8261
X_4 non-fat muscle wt. (g)	2.8023	2.2289	3.3109	3.0065	2.4304	3.4537	2.9577	2.4247	3.4203
X_5 muscle fat wt. (g)	0.5644	−0.0969	1.0843	0.8227	0.3422	1.3474	0.7061	0.2127	1.0566
X_6 non-fat liver wt. (g)	1.0996	0.6251	1.6308	1.2887	0.6866	1.8342	1.2387	0.5688	1.8545
X_7 liver fat wt. (g)	0.9892	0.4556	1.6934	1.3131	0.4684	1.9634	1.2053	0.3847	1.8258
Y_1 As−M (μg)	3.1651	2.6575	4.0469	3.2509	2.5755	3.8167	3.2597	2.1832	3.9344
Y_2 Cu−M (ng)	5.5333	4.8824	6.2677	5.6567	5.0878	5.9783	3.5014	5.0050	6.2775
Y_3 Hg−M (ng)	4.3877	3.6412	5.2581	4.7914	3.9397	5.4526	4.6865	3.8423	5.5167
Y_4 Zn−M (ng)	6.4290	5.8096	7.0054	6.6243	6.0690	7.0391	6.6249	6.0913	7.1790
Y_5 As−L (μg)	1.6461	1.1474	2.4612	1.8636	1.0880	2.4116	1.9109	1.3586	2.4768
Y_6 Cd−L (ng)	3.1368	2.3972	4.0470	3.8002	3.2083	4.4356	3.3862	2.6145	4.0834
Y_7 Cu−L (μg)	1.9541	1.2610	2.6157	2.2402	1.4583	2.8222	2.3655	1.4741	3.1520
Y_8 Hg−L (ng)	2.7100	2.1596	3.6545	3.0470	2.1931	3.6542	2.8538	2.0191	3.7900
Y_9 Se−L (ng)	4.2687	3.7500	4.9038	4.6428	4.0726	5.1021	4.6726	3.9222	5.3467
Y_{10} Zn−L (μg)	2.5394	2.0144	3.1461	2.8027	2.1550	3.2493	2.8061	2.1769	3.4314
Y_{11} PCB−L (μg)	1.6320	1.0652	2.5834	2.0834	1.2184	2.6964	1.9587	1.1946	2.8698
Y_{12} α−HCH−L (ng)	3.2183	2.5705	3.7351	3.4355	2.6702	4.0660	3.3545	2.5780	3.9656
Y_{13} HCB−L (ng)	2.9833	2.2610	4.0470	3.2791	2.3556	3.8516	3.1665	2.3495	3.7566

for deviations from this linear time trend and contrasts identifying individual contaminant−tissue combinations which contribute significantly to time trends. In the present study, we employed the union intersection (UI) approach to multivariate hypothesis testing, as it has the advantages of (a) leading directly to simultaneous confidence bounds on all double linear compounds $\boldsymbol{C}\theta\boldsymbol{D}$ where $\boldsymbol{C}$ is the $(g \times m)$ "hypothesis matrix" and $\boldsymbol{D}$ is a $(u \times v)$ matrix of rank $v \leq u$ and (b) providing a powerful test when the first characteristic root is much larger than the other roots (as was the case with the cod data analyzed here).

Analysis and Results

In the analyses reported here, all tests were carried out at the 5% probability level (P) of significance unless stated otherwise. Cod data were analyzed for possible outlying X and Y observations and the overall impact of an observation on the fitted MLR evaluated. The MLRs for concentration and burden models were examined for each of the 13 contaminant−tissue combinations. The following results reported.

(1) For MLR models (1) through (5), several observations outlying on X and Y were identified. Ranges of numbers of outlying observations were (a) for X observations, 9−12 (concentration models) and 7−10 (burden models), and (b) for Y observations, 2−8 (concentration and burden models).

(2) Fewer Y and X outliers were shown in model (6) than in the other five models which employed variable X_1 and/or its functions. For model (6), ranges of the number of outlying observations were (a) for X observations, 2−6 (concentration models) and 2−5 (burden models), and (b) for Y observations, 0−3 (concentration and burden models).

TABLE 3. Time trend vectors for adjusted mean values (concentration and burden data). *$P \leq 0.05$.

		Covariate												
		Concentration data							Burden data					
	Variable	X_2	X_3	X_4	X_5	X_6	X_7		X_2	X_3	X_4	X_5	X_6	X_7
Y_1	As–M	0.1004	0.0671	0.0652	0.0598	0.0641	0.0530		0.2059	0.1026	0.0651	0.0613	0.0461	0.0072
Y_2	Cu–M	0.1607	0.1618*	0.1667*	0.1832*	0.1664*	0.1881*		0.2662*	0.1971*	0.1667*	0.1637	0.1426	0.1251
Y_3	Hg–M	0.0015	−0.1611	−0.0765	−0.1352	−0.0877	−0.1557		0.1069	−0.0256	−0.0766	−0.0850	−0.1175	−0.1609
Y_4	Zn–M	−0.0345	−0.0412	−0.0408	−0.0401	−0.0412	−0.0421		0.0710	−0.0059	−0.0408	−0.0475	−0.0668	−0.0892
Y_5	As–L	−0.0432	−0.0588	−0.0637	−0.0762	−0.0840	−0.0861		−0.0142	−0.0903	−0.1221	−0.1191	−0.1293	−0.1149
Y_6	Cd–L	−0.0037	−0.0262	−0.0334	−0.0572	−0.0793	−0.0827		0.0253	−0.0578	−0.0918	−0.0919	−0.1098	−0.1225
Y_7	Cu–L	−0.1651	−0.1980	−0.2006	−0.2199	−0.2159	−0.2323		−0.1362	−0.2296	−0.2589	−0.2588	−0.2641	−0.2490
Y_8	Hg–L	0.1913	0.1222	0.1062	0.0548	0.0599	0.0105		0.2202	0.0906	0.0478	0.0431	0.0322	−0.0053
Y_9	Se–L	−0.1592	−0.1960*	−0.1997*	−0.2115*	−0.2319*	−0.2370*		−0.1302	−0.2275*	−0.2582*	−0.2599*	−0.2635*	−0.2754*
Y_{10}	Zn–L	−0.0515	−0.0729	−0.0741	−0.0772	−0.0994	−0.0946*		−0.0226	−0.1045	−0.1324	−0.1318	−0.1349*	−0.1354
Y_{11}	PCB–L	−0.0280	−0.0680	−0.0848	−0.1268	−0.1081	−0.1500		0.0010	−0.0994	−0.1431	−0.1506	−0.1606	−0.1489
Y_{12}	α–HCH–L	0.0054	0.0274	0.0328	0.0460	0.0591	0.0749		0.0344	−0.0042	−0.0258	−0.0180	−0.0170	0.0652
Y_{13}	HCB–L	−0.0058	0.0047	0.0031	0.0039	0.0233	0.0269		0.0232	−0.0269	−0.0553	−0.0523	−0.0535	0.0259

(3) A concentration model did not yield exactly the same Y and X outliers as its corresponding burden model.

(4) There were, however, only three outlying observations (one in each year) shown by Cook's statistic which were extreme enough to have distorting effects on the estimated regression coefficients. No biologically and/or chemically based explanation for the occurrence of these outliers was found. Five observations were identified in the "gray" zone (Cook's analysis) by concentration and/or burden model (6).

At this time, no attempt was made to explore data in search of the "best" MLR equation. In the MANCOVA, we employed only one covariate by considering the biological parameters one at a time. This was done in view of the fact that these biological parameters are key variables and as such are individually important. In all MANCOVA's, (a) the three fish samples earlier identified as distinct outliers were excluded from data sets and (b) fish samples analyzed were all "complete", i.e. for which all 19 measurements (13 contaminant–tissue combinations and six biological parameters) were available. Missing observations virtually destroy multivariate analyses (Pimentel 1979). After removing the outliers, there were 33, 44, and 44 fish samples left from 1977, 1978, and 1979, respectively. Table 2 shows means and ranges of contaminant–tissue combinations and biological variables for concentration and burden data, respectively, of individual years.

In the following discussion, MANCOVA is explained at length for one case only, viz. concentration data set for contaminant–tissue combinations $Y_{i1},\ldots,Y_{i13}$ and covariate X_{i2}. All other analyses were done in exactly the same way. Following Bliss (1970), it was noted that ranges of Y_{ik} ($k = 1,\ldots,13$) overlapped enormously between years (Table 2). Bliss (1970, p. 332) explained the difficulty in retrieving meaningful interpretations from univariate analyses when such overlappings occur. We illustrate this by considering, as an example, data for 1977 and 1978. Between these two years, ranges overlapped from 61% (Y_6) to 100% (Y_2, Y_5, Y_9, and Y_{10}) in 1977 and from 42% (Y_2) to 100% (Y_4) in 1978. With these large overlaps, no single Y would separate the years effectively. Yet, univariate ANOVA rejected the null hypothesis of equality of means for the two years in six contaminants (Y_1, Y_2, Y_3, Y_6, Y_9, and Y_{11}), P being very small in some cases (e.g. for Y_3, $F = 20.71$ with df = 1,118). These findings indicate, in yet another way, the necessity of employing a compound criterion (a multivariate approach) of contaminant–tissue combinations. The null hypothesis of no difference among the three years in their slopes (weighted by sums of squares of the covariate) was tested and accepted ($P > 0.70$). The hypothesis $\boldsymbol{B} = 0$, where $\boldsymbol{B}$ is the matrix of common slope coefficients computed from within year or error sums of squares and product matrices (Morrison 1976, p. 195), was rejected ($P = 0+$, i.e close to zero), indicating thereby that the years should be compared and time trends examined on the basis of means of Y_{ik} "adjusted" (by MANCOVA) for inequalities among individuals in the values of their covariate. The hypothesis of equal vectors of adjusted means of Y_{ik} was rejected ($P = 0+$), indicating thereby that the three years differed in their mean values of one or more contaminant–tissue combinations, or a linear combination of these. Following the rejection of the hypothesis for adjusted mean vectors of the three years, time trend was examined by defining the year contrast the same way as Scott et al. (1983) did, i.e. as −1, 0, and 1 for 1977, 1978, and 1979, respectively. This time trend was significant ($P =$

0+). Ninety-five percent simultaneous confidence intervals for individual contaminant–tissue combinations showed that the linear time trend did not exist for any individual contaminant–tissue combination. The contrast for deviations from the linear trend was also significant. Several years of data would obviously be required to establish the true mathematical form of the underlying pattern. This analysis indicated that even if the true pattern is nonlinear, a significant part of it was captured by the linear time trend.

Findings for the other 11 MANCOVA's (i.e. five for the concentration data and six for the burden data) were similar to those of the MANCOVA presented above, except that in a number of cases Y_2, Y_9, and Y_{10} did contribute significantly to linear time trends. Table 3 shows time trend vectors for adjusted mean values. Each trend vector is upward for some variables and downward for others.

We conclude that (1) for the present data set where dependent variables are highly correlated mutually, univariate analyses (ANOVA and MLR) would have been ineffective for examining differences among years in general and for time trends in particular and that (2) linear time trends in cod data were clearly indicated even though these did not explain all variations over years.

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Stock Identification with the Maximum-Likelihood Mixture Model: Sensitivity Analysis and Application to Complex Problems

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Wood, C. C., S. McKinnell, T. J. Mulligan, and D. A. Fournier. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44: 866–881.

Simulations were performed to evaluate the bias and precision of stock composition estimates from the maximum-likelihood mixture model using hypothetical multilocus characters. Bias and precision were examined in relation to the number of stocks being resolved, the number of loci available, and the difference in allelic frequency among stocks at each locus, using Monte Carlo simulations with different levels of sampling error in the mixture and learning samples. Model performance improved with increasing stock separation and number of loci available. Bias was not affected by the number of stocks resolved in simulations where mixture contributions from individual stocks remained constant. These results provide guidelines for reducing the complexity of genetic stock-identification problems by summing estimated mixing proportions for individual stocks within groupings based on stock similarity. The trade-off between improved accuracy and level of grouping can be examined graphically to determine the most useful level of grouping for the problem at hand. We illustrate this procedure with a real example from mixed-stock fisheries on sockeye salmon (*Oncorhynchus nerka*) along the British Columbia – Alaska coast.

Les auteurs ont effectué des simulations afin d'évaluer le biais et la précision des estimations de la composition du stock tirées d'un modèle de la vraisemblance maximum du mélange élaboré à partir de caractéristiques hypothétiques des multi-locus. Le biais et la précision ont été étudiés en fonction du nombre de stocks examinés, du nombre de locus disponibles et de la différence entre les fréquences alléliques chez les stocks à chaque locus. Ils ont utilisé des simulations Monte Carlo à divers niveaux d'erreur d'échantillonnage dans les échantillons d'apprentissage et les échantillons complexes. Le rendement du modèle s'est amélioré en fonction d'un accroissement de la séparation des stocks et du nombre de locus disponibles. Le biais ne variait pas selon le nombre de stocks examinés par simulation où les contributions complexes de stocks individuels demeuraient constantes. Les résultats obtenus serviront de lignes directrices pour réduire la complexité des problèmes d'identification génétique de stock : ainsi, on obtient la somme des proportions estimatives du mélange pour chaque stock à l'intérieur de regroupements basés sur la ressemblance des stocks. L'équilibre entre le gain de précision et le niveau de regroupement peut être étudié à l'aide de graphiques afin de déterminer le meilleur niveau de regroupement dans le cas d'un problème particulier. Les auteurs appliquent cette méthode à la pêche pluristocks du saumon rouge (*Oncorhynchus nerka*) effectuée le long du littoral de la Colombie-Britannique et de l'Alaska.

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Many fisheries exploit mixtures of fish from several stocks. Wherever it is desirable to manage these mixed stocks independently owing to differences in productivity, for example, fishery managers will require efficient techniques for stock identification. Although a wide variety of biological characteristics have been used for stock identification, the analytical procedures used to estimate mixture composition fall into one of two basic categories: classification models (Worlund and Fredin 1962; Cook and Lord 1978; Pella and Robertson 1979) and mixture models (Milner et al. 1981, 1983; Fournier et al. 1984). The latter approach is more versatile for mixed-fishery problems because both the sample distribution of phenotypes and the characteristics of individual fish provide clues to the mixture composition, and because incomplete data for mixture samples can be used. In fact, the classification-with-correction approach (e.g. Pella and Robertson 1979) is a special case of the maximum-likelihood solution where some information about mixture composition is lost (see Appendix; also Millar 1987). Fournier et al. (1984) demonstrated that the maximum-likelihood solution of the mixture model is unique and asymptotically unbiased. There are, however, several sources of error that must be considered in practical applications (Fig. 1).

The most important source of error involves the choice of reference stocks and the uniqueness of the learning samples for these stocks. Obviously, if learning samples are not available for one or more stocks present in a mixture sample, the estimates of mixture composition will be biased. Similarly, if a composite sample representing several substocks is considered as a single learning sample, one must ensure that the composite learning sample reflects the relative magnitude of contributions by the individual substocks to the mixture sample. The size of the mixture sample required to provide satisfactory estimates of composition will depend on the number of stocks contributing

[1] Appendix

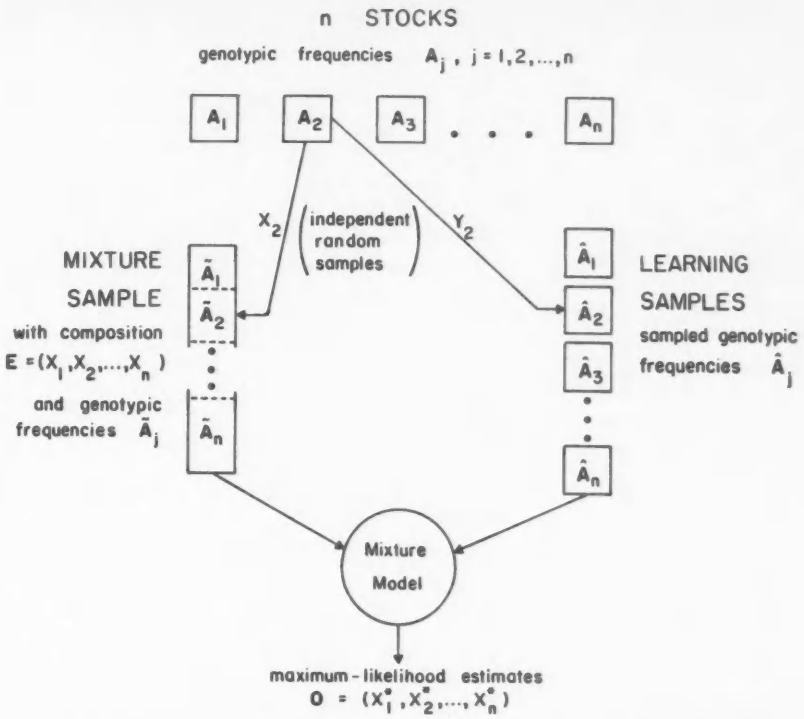


FIG. 1. Potential sources of error in stock composition estimates using the maximum-likelihood mixture model. Monte Carlo simulations were used to evaluate estimates for mixtures of known composition E. First, perfect mixtures and learning samples ($A_j = \hat{A}_j = \tilde{A}_j$ for all j) were generated to test for errors in the mixture model algorithm. Subsequently, random sampling errors were introduced to both the mixture and learning samples to evaluate the model's performance in practical situations. In all simulations, the A_j (but not the $\hat{A}_j$ or $\tilde{A}_j$) conformed to a Hardy–Weinberg (multinomial) distribution.

to the mixture, on the size of the learning samples, on how closely the learning samples resemble one another (i.e. stock separation), and on the number of useful characters being examined. For classification techniques with multilocus genetic characters, Smouse et al. (1982) have shown that the probability of correct allocation of individuals increases with stock separation and the number of traits examined, but decreases as the number of candidate populations increases.

Our objective was to examine the performance of the mixture model (specifically, that described by Fournier et al. 1984) using simulated multilocus genetic characters. We evaluated the influence on bias and precision of stock separation, number of loci, number of stocks, and the size of the mixture and learning samples. Based on these results, we suggest guidelines for practical application of the method. A real example from mixed-stock fisheries on sockeye salmon (*Oncorhynchus nerka*) along the British Columbia – southeast Alaska coast is solved using both genetic and nongenetic characters to illustrate the recommended procedure.

Methods

Monte Carlo Simulations Using Genetic Characters

Correct estimation of the component stocks of a mixture depends on the amount of information about stock differences available to the model; without adequate information, bias and reduced precision result. We used a factorial design for our simulations with genetic data to investigate the effect of varying stock separation at each locus, the number of loci, the number of stocks being resolved, and the size of the mixture and learning samples. A total of 624 different mixture problems were solved at unique combinations of these five factors (Table 1). The relative stock composition of mixture samples was held constant during resampling in these simulations. This was intentional so that we could measure the error inherent in the

TABLE 1. Factorial design for Monte Carlo simulations where all stocks contributed equally to the mixtures. Some combinations of factors could not be simulated; the maximum number of stocks considered was seven and five at a separation of 0.15 and 0.20, respectively.

Factor	Values in simulations
Stock separation at each locus (P_{ij})	0.05, 0.10, 0.15, 0.20
Number of loci	3, 6, 9
Number of stocks	3, 5, 7, 9
Mixture sample size (fish/stock)	10, 25, 50, 100
Learning sample size	10, 25, 50, 100

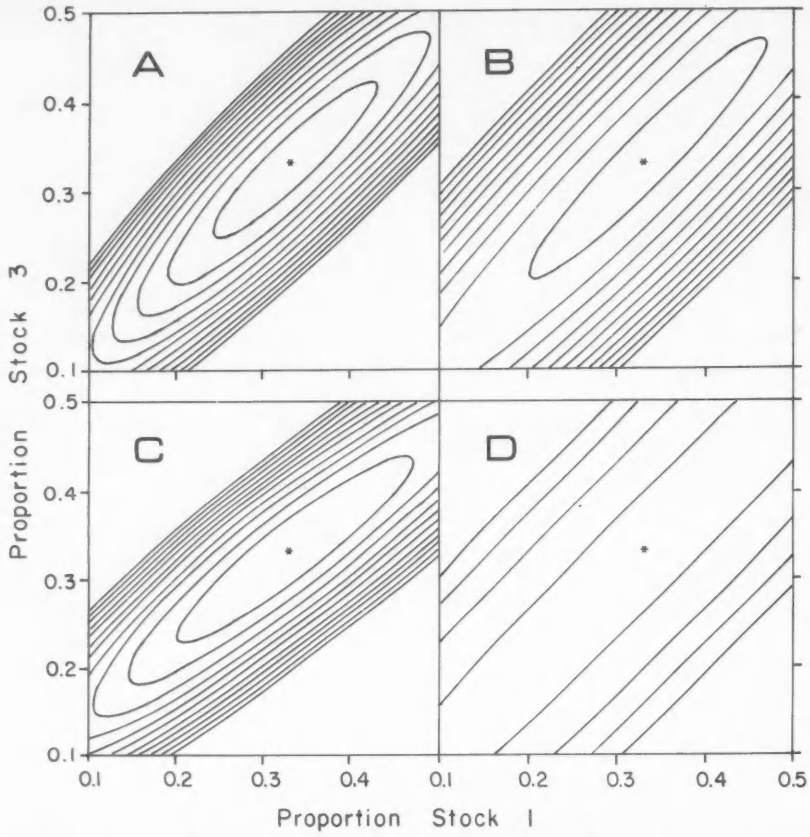


FIG. 2. Comparison of stock separation indices for three-stock mixture problems without sampling error (i.e. "infinite sample size") where each stock contributed equally. The allelic frequency of the median stock (p_2) was 0.12 in mixtures A and C versus 0.50 in mixtures B and D. The contour plots represent the resulting log-likelihood surfaces for each mixture where the maximum-likelihood estimates (0.333 for each stock) are represented by asterisks; contour intervals are equal in all diagrams to allow direct comparison of gradients. Mixture problems with fixed differences in allelic frequency between stocks (P_{ij}) were of comparable difficulty whether $p_2 = 0.12$ or $p_2 = 0.50$ whereas mixture problems with a fixed Nei distance (D_{ij}) were relatively more difficult with $p_2 = 0.50$ (since the log-likelihood surface is flatter). In A and B, allelic frequencies p_1 and p_3 were specified by $p_2 - P_{12}$ and $p_2 + P_{23}$, respectively, where $P_{12} = P_{23} = 0.10$ (i.e. 0.02 and 0.22 in A and 0.40 and 0.60 in B). In C and D, p_1 and p_3 were defined such that the genetic distance $D_{12} = D_{23} = 0.006641$ (i.e. 0.02 and 0.203842 in C and 0.442184 and 0.557816 in D). Note that $P_{12} = 0.10$ is equivalent to $D_{12} = 0.006641$.

estimation procedure, not in the combination of estimation procedure and mixture sample. Real-world problems will, of course, include both sources of error.

Creating a Simulated Stock

Simulated stocks were assigned characteristic allelic frequencies at nine loci. All loci for a particular stock had identical allelic frequencies, but each locus segregated independently. Each locus had two alleles determining three genotypes in Hardy–Weinberg (binomial) proportions. For example, if the frequency of the first allele was $p = 0.3$, the second allele was $q = 1.0 - p = 0.7$ and the expected genotypic frequencies for all loci were given by the terms of the binomial expansion $p^2 + 2pq + q^2$ (i.e. 0.09, 0.42, 0.49). Thus, the expected (multilocus) genotypic frequency distribution for a particular stock was completely specified by the frequency of one allele (p).

Observed (i.e. sampled) genotypic distributions were generated by random sampling from the frequency distribution of genotypes using the IMSL (International Mathematical and Statistical Library) routine GGBN. Observed genotypic distributions were generated independently at each locus. Collectively, these sampled genotypic distributions are referred to as the "learning samples" and are used to estimate the true genotypic frequencies, A_j, for each stock j. Learning sample sizes were set at 10, 25, 50, or 100 fish/stock.

Measuring Stock Separation at Each Locus

Mixtures of very similar stocks are difficult to resolve; in the extreme, two identical stocks can never be resolved. Thus, a

stock separation measure was required to assess the degree of similarity, or conversely, separation between stocks. Two indices of stock separation were compared to determine which measure was most suitable for representing stock separation over a range of allelic frequencies. The first trial index, Nei's genetic distance (Nei 1972) between two stocks i and j at a single locus, is given by

$$(1)\quad D_{ij} = -\log_e \frac{\sum_l p_{il}p_{jl}}{\sqrt{\left[\sum_l p_{il}^2 \sum_l p_{jl}^2\right]}}$$

where p_{il} is the frequency of allele l in stock i. For the experiments with simulated genetic data with only two alleles, the p_{il} were specified by p_i and $1 - p_i$. The second trial index was simply the difference in the frequency of one allele, $P_{ij} = p_i - p_j$ for stocks i and j.

The log-likelihood surface for three-stock mixture problems shown in Fig. 2 with respect to proportions of two of the stocks was computed for problems involving constant increments of D_{ij} and P_{ij} at low allelic frequencies (median stock centered at 0.12) and intermediate allelic frequencies (centered at 0.50). The contour plots presented in Fig. 2 demonstrate that for a given Nei distance, the mixture problem becomes progressively more difficult (i.e. the surface becomes flattened) as the allelic frequencies tend toward 0.50. Thus, D_{ij} was considered unsatisfactory for the specific purpose of these simulation analyses whereas P_{ij} provided a satisfactory index for allelic frequencies ranging from 0.02 to 0.50. Accordingly, four levels of stock separation ($P_{ij} = 0.05, 0.10, 0.15, 0.20$) were specified to generate four sets of learning samples for the nine candidate stocks. (Note that the maximum P_{ij} for a seven-stock problem was 0.15 and for a nine-stock problem was 0.10, as all allelic frequencies must lie in the range 0−1.)

Mixtures

Mixtures of three, five, seven, and nine stocks were generated for different levels of stock separation. In each case, the median stock was centered at an allelic frequency of 0.50. Mixture samples were created independently of the learning samples by randomly drawing genotypes at three, six, or nine loci from the genotypic distribution characteristic of each contributing stock. Except where stated, the number of fish from each candidate stock was always equal (i.e. 10, 25, 50, or 100 fish/stock). Overall mixture size depended on the number of stocks contributing but ranged from 30 to 900.

Simulations without Sampling Error

The mixture model is asymptotically unbiased (Fournier et al. 1984). Given an infinitely large sample size such that the genotypic frequencies contributed by each stock to the mixture are exactly as expected, estimates of stock proportions in the mixture should be exactly correct. Of course, sampling error is to be expected with finite sample sizes and this will usually result in increased bias and variance of the estimates. To verify that our mixture model algorithm was functioning correctly, we generated mixtures and learning samples without sampling error for two three-stock problems. In the first problem, allelic frequencies for each stock were set at $p_1 = 0.40$, $p_2 = 0.50$, and $p_3 = 0.60$ (i.e. $p_{ij} = 0.10$) at each of three independent loci; in the second problem, $p_1 = 0.30$, $p_2 = 0.50$, and $p_3 = 0.70$ (i.e. $p_{ij} = 0.20$) at each of nine independent loci. The stock composition of each mixture was identical with proportions of 0.0, 0.50, and 0.50 for stocks 1, 2, and 3, respectively. (The actual size of the mixture and learning samples was 200 and 100, respectively, but all genotypic frequencies were exactly as expected for the binomial distributions specified by p_1, p_2, and p_3.)

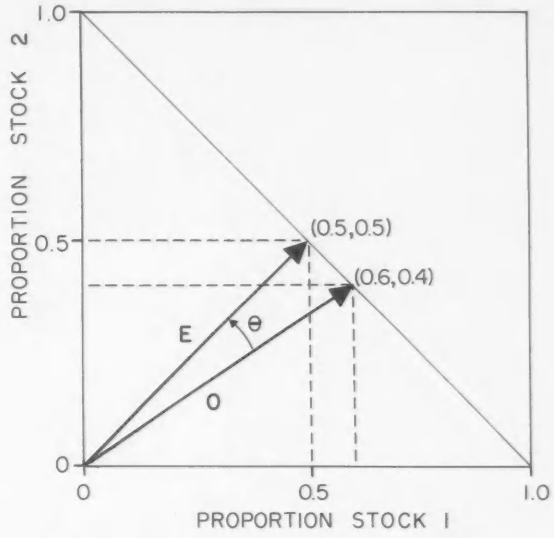


FIG. 3. Angular deviation (θ) of the estimated mixture composition vector $\boldsymbol{O}$ = (0.6, 0.4) from the true mixture composition vector $\boldsymbol{E}$ = (0.5, 0.5) for a two-stock mixture problem.

Measuring Model Performance with Sampling Error

To evaluate the effect of sampling error on the model's performance, we required a suitable index that would reflect the tendency of the estimates of mixture composition to deviate from the actual composition. If s is the number of stocks in the problem, the true mixture composition can be thought of as a vector $\boldsymbol{E}$ of order s whose elements constitute the expected proportions of each stock (Fig. 1). A vector $\boldsymbol{O}$ of similar order is defined as the estimated proportion from one simulation run. If, after repeated simulations, mean estimates do not tend toward the expected result, they are biased in some way. The difference between the expected result and the estimated composition was measured as the angle between the two vectors in s-dimensional space:

$$(2)\quad \text{Angular deviation for simulation } m \text{ (in radians)} = \theta_m = \cos^{-1}\left(\frac{\boldsymbol{E}\cdot\boldsymbol{O}}{\|\boldsymbol{E}\|\,\|\boldsymbol{O}\|}\right).$$

Each mixture type and its corresponding learning samples were generated 50 times (except where stated) using the Monte Carlo procedures already described to introduce sampling error. Overall performance was measured by calculating the mean angular deviation over n runs:

$$(3)\quad \text{Mean angular deviation} = \bar{\theta} = \frac{1}{n}\sum_{m=1}^{n}\theta_m.$$

Figure 3 illustrates a simple two-stock problem where the expected result is (0.5, 0.5), i.e. 50% each stock. The solution

TABLE 2. Simulation results using allocate-sum and pool-allocate clustering procedures to simplify a difficult mixture problem. Means calculated from estimates from 200 simulations; numbers in parentheses represent 1 SD. * 95% C.I. for mean does not include true value.

Mixture type	Stock	Allelic frequency	True proportions in mixture		Estimated proportions		
					Allocate-sum		Pool-allocate
			By stock	By group	By stock	By group	
Equal	1	0.10	0.111		0.105		
	2	0.15	0.111	0.333	0.115	0.333	0.342*
	3	0.20	0.111		0.113	(0.014)	(0.008)
	4	0.40	0.111		0.112		
	5	0.50	0.111	0.333	0.107	0.335	0.308*
	6	0.60	0.111		0.116	(0.037)	(0.019)
	7	0.70	0.111		0.113		
	8	0.73	0.111	0.333	0.116	0.332	0.350*
	9	0.76	0.111		0.103	(0.036)	(0.015)
Random	1	0.10	0.104		0.103		
	2	0.15	0.138	0.365	0.133	0.368	0.379*
	3	0.20	0.123		0.132	(0.016)	(0.009)
	4	0.40	0.156		0.140		
	5	0.50	0.102	0.411	0.137	0.389*	0.368*
	6	0.60	0.152		0.112	(0.034)	(0.018)
	7	0.70	0.069		0.093		
	8	0.73	0.051	0.224	0.080	0.243*	0.253*
	9	0.76	0.104		0.070	(0.032)	(0.015)
Uneven	1	0.10	0.0		0.000		
	2	0.15	0.0	0.0	0.000	0.000	0.000
	3	0.20	0.0		0.000	(0.000)	(0.000)
	4	0.40	0.0		0.001		
	5	0.50	0.0	0.9	0.163	0.757*	0.556*
	6	0.60	0.9		0.593	(0.072)	(0.034)
	7	0.70	0.1		0.194		
	8	0.73	0.0	0.1	0.043	0.244*	0.444*
	9	0.76	0.0		0.007	(0.072)	(0.034)

vector, derived from one simulation run, is (0.6, 0.4) and the deviation from the expected vector is summarized by the angle θ. An estimate exactly equal to the expected yields $\theta = 0.0$. Similarly, the mean angle, $\bar{\theta}$, was used to evaluate model performance in repeated simulations with sampling error under each experimental condition. Typically, θ ranged from 0.1 to 0.5 radians in our results. Except where stated, all simulated mixtures comprised equal contributions from each stock. Therefore, the worst possible deviations would have resulted from allocation of the entire mixture to one stock, resulting in maximum values of θ between 0.9 for three stocks and 1.3 for nine stocks. Because typical values were much less than maximum values, we have ignored the influence of number of stocks on this index.

Measuring Model Performance with Clustering of Stocks

Mixture resolution problems that are too difficult to solve directly owing to inadequate separation between a large number of stocks can be simplified by grouping similar stocks together (e.g. Pella and Milner 1987; Millar 1987). We evaluated two alternative clustering procedures: in the first, similar stocks were pooled together to produce a reduced number of composite learning samples for the mixture model; in the second, the mixture model was used to estimate contributions by all component stocks which were summed within groupings of similar stocks to provide a reduced number of estimates. We refer to the former as the "pool-allocate" procedure and the latter as the "allocate-sum" procedure. The pool-allocate procedure requires additional knowledge about the relative contribution of each individual stock to the mixture sample; ideally, the composite learning sample should consist of pooled stocks each weighted by their relative abundance in the mixture. The degree to which this a priori choice of stock contribution influences the characteristics of the composite stock depends on the similarity of the stocks which are pooled.

To evaluate the merits of each clustering procedure, nine new stocks were simulated, as before, but with asymmetrical stock separation resulting in three natural clusters of similar stocks. A similarity dendrogram was calculated by the single linkage method (Pielou 1977) using a pair-wise matrix (9×9) of distance measures defined by

$$(4)\quad \text{Distance between stock } i \text{ and stock } j = \phi_{ij} = \left[\sum_{k=1}^{M} D_{ijk}^2\right]^{1/2}$$

where $D_{ijk} = D_{ij}$ in equation (1) for locus k and $k = 1, \ldots, M$. The distance measure ϕ_{ij} represents the Euclidean distance over M independent loci, rather than the geometric mean. This modification of Nei's genetic distance (Nei 1972) was necessary because we require an index of separation potential, given the available information, rather than an index that reflects overall genetic divergence of populations. It is easy to show that distances calculated with (4) increase as additional independent but identically distributed loci are added whereas dis-

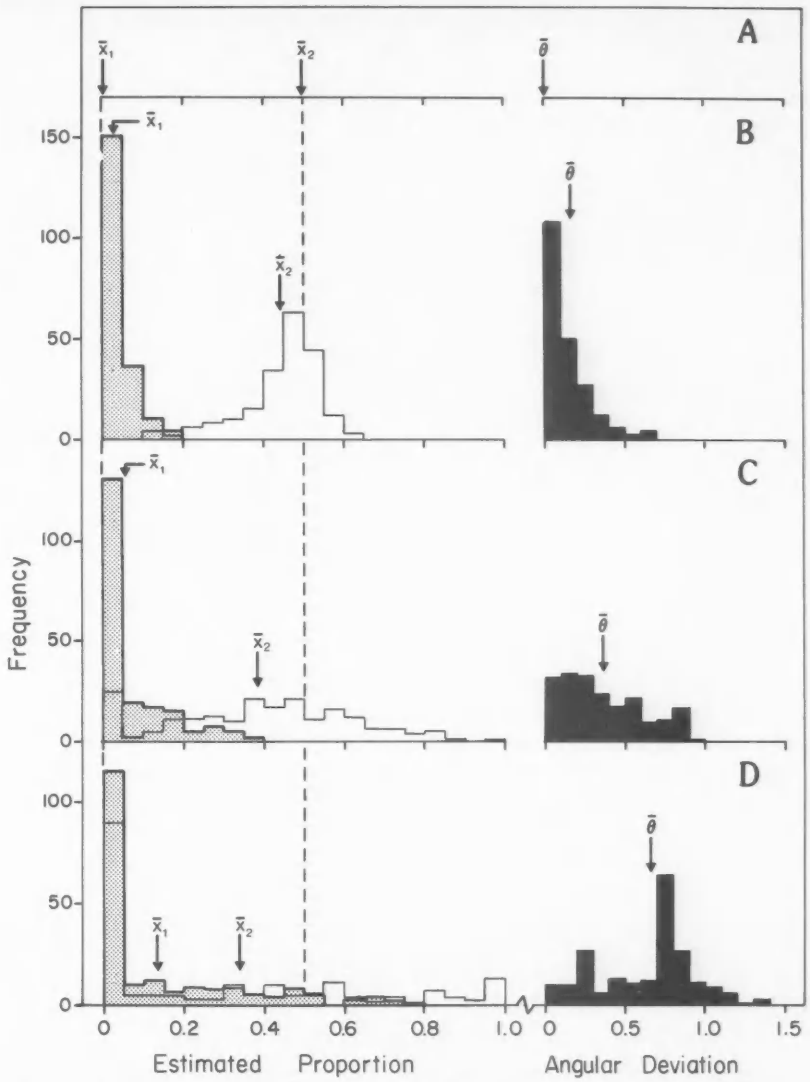


FIG. 4. Effect of mixture sampling error on the distributions of the estimated stock proportions and the angular deviation index for a "difficult" problem ($P_{ij} = 0.10$ at three loci). All mixtures were composed of three stocks in the proportions 0.0, 0.50, and 0.50 with (A) no sampling error, or mixture samples sizes of (B) 2000, (C) 200, and (D) 20. No sampling error was introduced to the learning samples. The distributions for stock 3 are not presented but resemble those for stock 2.

tances calculated by combining loci as suggested by Nei (1972) do not.

Stock composition of known mixtures was estimated using both the allocate-sum and pool-allocate procedures. Three mixtures of 900 fish each and differing in stock composition were defined: the first mixture comprised equal proportions from all nine stocks (100 fish each); the second mixture comprised all nine stocks in proportions selected randomly which varied between 0.051 and 0.156 (Table 2); the third mixture comprised only two stocks (numbers 6 and 7) in proportions of 0.90 and 0.10, respectively. Stock composition was estimated 200 times for each mixture. Although stock composition was known exactly, sampling variability was introduced independently to genotypic frequencies in both the mixture and learning samples during each simulation as described for previous experiments.

Results

Sensitivity Analysis

The typical effect of sampling error on the distribution of mixture composition estimates is illustrated in Fig. 4 and 5 for mixtures composed of three stocks in proportions 0.0, 0.5, and 0.5. We began with mixtures and learning samples generated

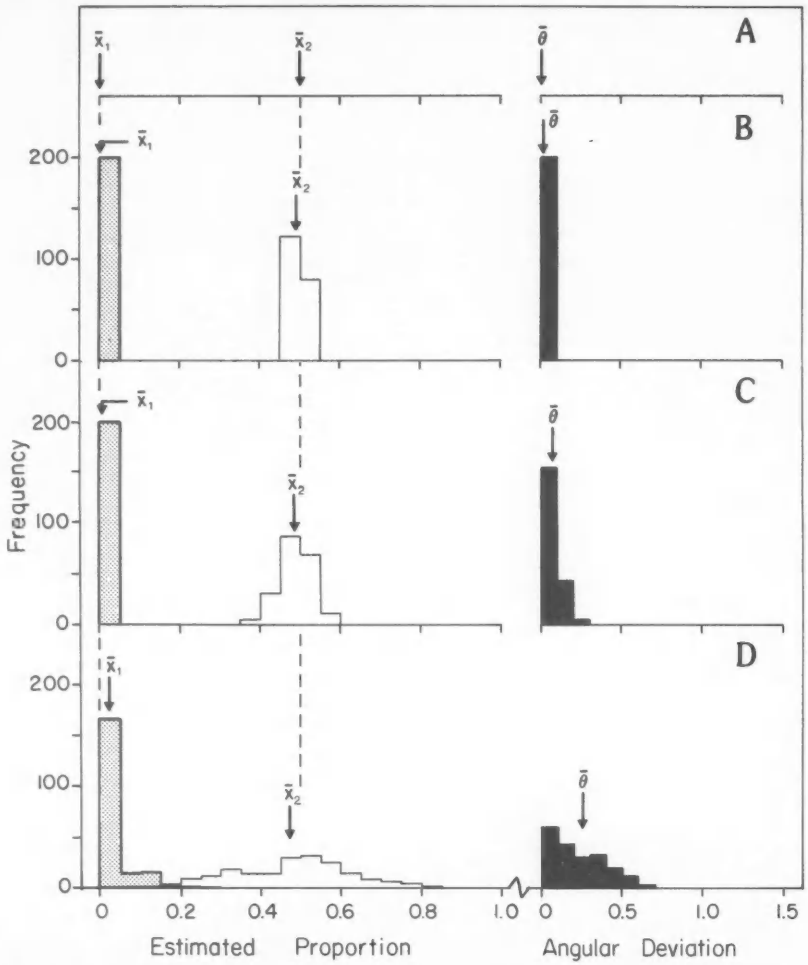


FIG. 5. Effect of mixture sampling error on the distributions of the estimated stock proportions and the angular deviation index for an "easy" problem (P_{ij} = 0.20 at nine loci). All mixtures were composed of three stocks in the proportions 0.0, 0.50, and 0.50 with (A) no sampling error, or mixture samples sizes of (B) 2000, (C) 200, and (D) 20. No sampling error was introduced to the learning samples.

without sampling error (see Methods) to mimic infinitely large samples. Less information was available for estimating mixture composition in Fig. 4 (P_{ij} = 0.10, three loci) than in Fig. 5 (P_{ij} = 0.20, nine loci). As expected, all estimated proportions were within ±0.03% of the true values. Next, we generated progressively smaller mixture samples (2000, 200, and 20) with identical stock proportions. (Learning samples were generated without sampling error.) As sample size decreased, estimates became biased and precision also decreased. In general, with finite mixture samples, the model underestimated contributions by dominant stocks and overestimated contributions by minor stocks. For stock proportions close to the constraints (0.0 or 1.0), the distributions of the estimates were highly skewed; for stock proportions near 0.5, the distributions of estimates were approximately normal.

The relationship between overall model performance measured by $\bar{\theta}$ to the bias and precision of estimates for individual stocks is also illustrated in Fig. 4 and 5. An advantage of $\bar{\theta}$ is that it summarizes the performance of the model over all stocks and greatly simplifies the task of presenting results. Accordingly, we have used this index to illustrate the general features of model performance under the various experimental conditions. It is not possible to evaluate accuracy and precision of the estimates independently with this index; however, accuracy and precision typically increase together as the angular deviation decreases.

The effect of learning sample size is illustrated in Fig. 6 for the three-stock problems just described, with mixture sample size fixed at 200. Again, bias increased and precision decreased as learning samples were diminished, but the effect is only noticeable for unrealistically small samples (<50 fish/stock). In most cases, learning samples corrected to Hardy–Weinberg equilibrium gave slightly better results, particularly for estimating the contribution by stock 1 which was never present in the mixtures.

The number of stocks in a mixture had little or no effect on

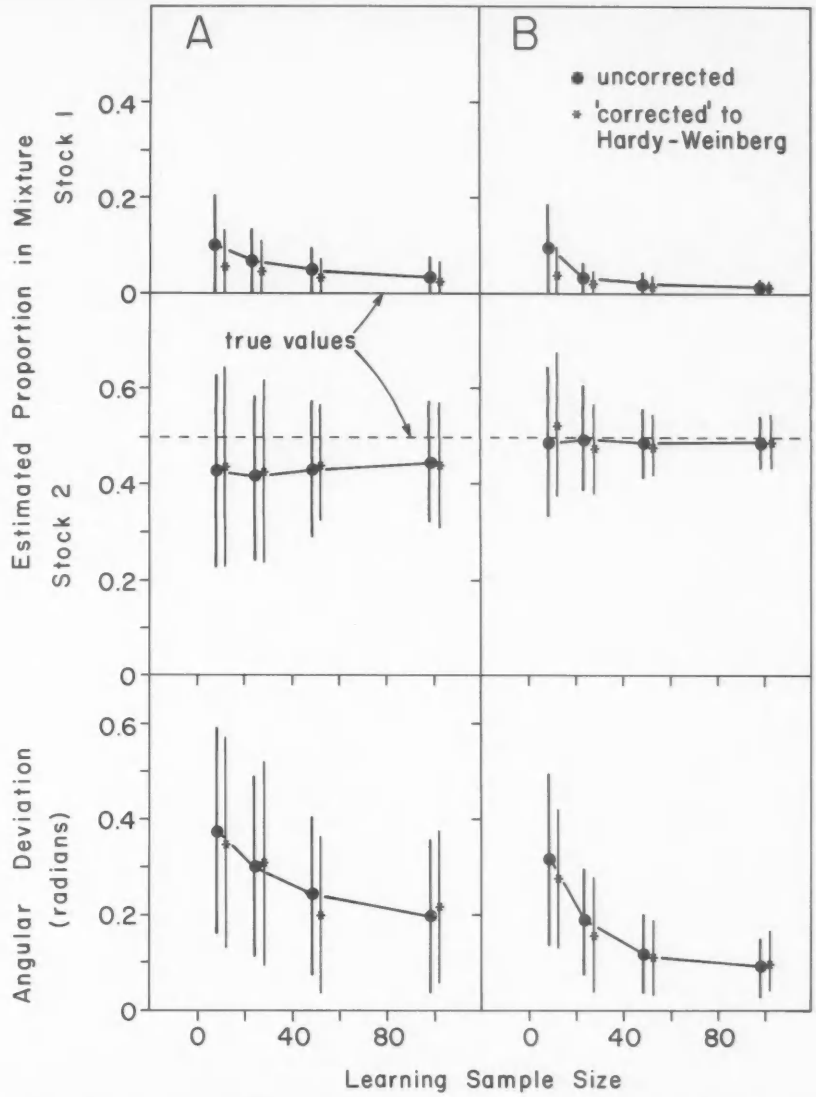


FIG. 6. Effect of learning sample size on mean estimated mixing proportions and angular deviations (±1 SD) for the mixture problems with mixture sample sizes of 200 in Fig. 4 and 5. (A) Difficult problem with $P_{ij} = 0.10$ at three loci; (B) easier problem with $P_{ij} = 0.20$ at nine loci. Asterisks indicate results obtained after "correcting" genotypic frequencies in the learning samples to those expected at Hardy–Weinberg equilibrium.

model performance although the number of fish per stock is important. This is illustrated by the perspective plot in Fig. 7 where the dependent variable is the mean angular deviation index $\bar{\theta}$. All stocks contributed equally to the mixture and learning sample sizes were fixed at 100 fish/stock. Except for the data points at three stocks and 100 fish/stock, the $\bar{\theta}$ surface is almost independent of the number of stocks in the mixture. A critical sample size of about 40 fish/stock is apparent, below which errors in estimation increase dramatically and above which errors remain relatively constant.

Because the number of stocks in the mixture had a negligible influence on the precision and accuracy of the estimates, we have simplified the presentation of subsequent results by ignoring differences in the number of stocks resolved. Results from mixtures composed of different numbers of stocks have been pooled together, provided the number of fish contributed by each stock was identical. Both learning and mixture sample sizes were observed to have critical values below which model errors increased dramatically (Fig. 8). Sample sizes greater than these critical values reduced the error, but the slope of the $\bar{\theta}$ surface is much less, especially along the learning sample size axis. Thus, for the sake of clarity, we will now consider

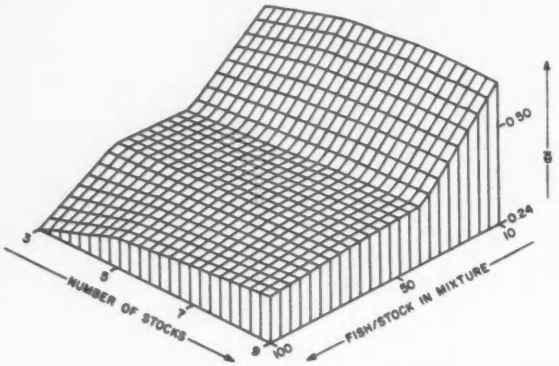


FIG. 7. Perspective plot of the interpolated $\bar{\theta}$ surface showing the effects of the number of stocks and the number of fish per stock in the mixture sample. The number of loci was fixed at nine, the separation between stocks at $P_{ij} = 0.10$, and the learning sample sizes at 100 fish/stock. High values of $\bar{\theta}$ indicate that stock composition estimates are both biased and imprecise.

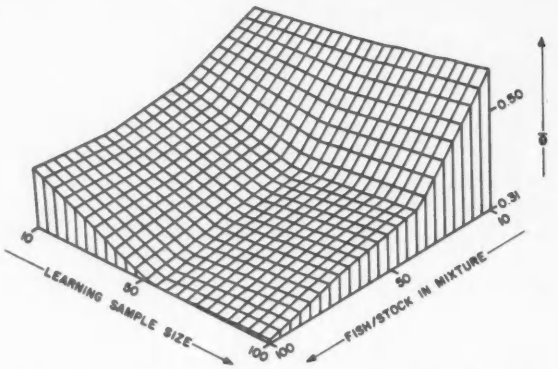
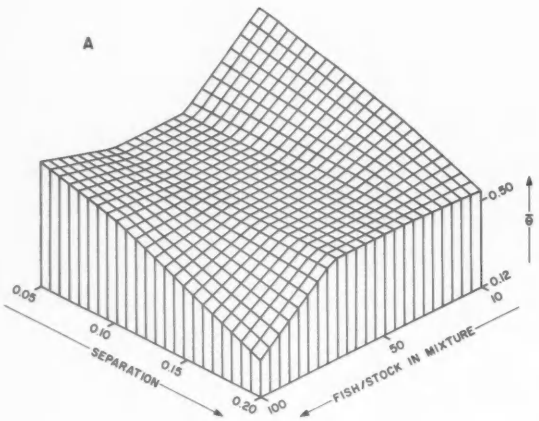


FIG. 8. Perspective plot of the interpolated $\bar{\theta}$ surface showing the relative effect of the number of fish per stock in the mixture versus the learning samples. The number of loci was fixed at nine and the separation between stocks at $P_{ij} = 0.10$.



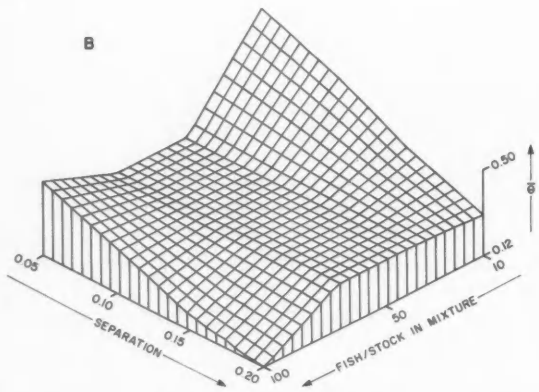
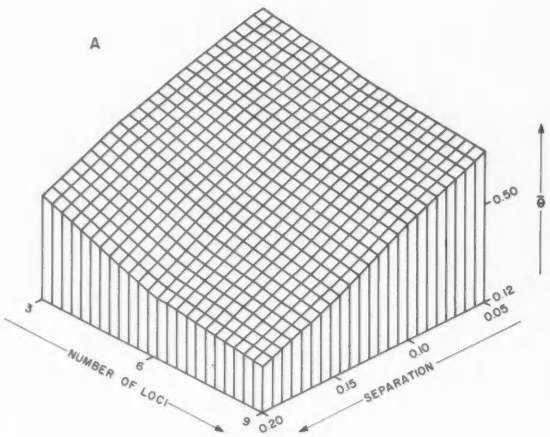


FIG. 9. Perspective plots of the interpolated $\bar{\theta}$ surface showing the effects of separation between stocks, P_{ij}, and the number of fish per stock in the mixture. The number of loci was fixed at (A) three and (B) nine; learning sample size was fixed at 100.



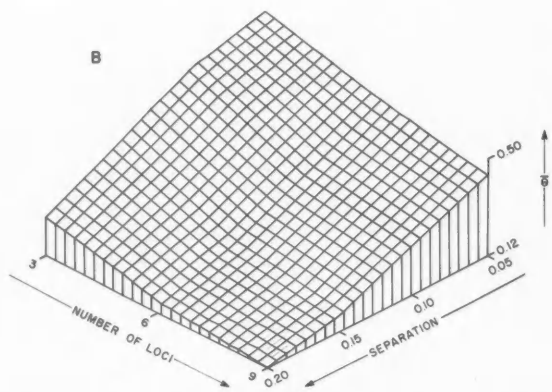


FIG. 10. Perspective plots of the interpolated $\bar{\theta}$ surface showing the effects of number of loci and separation between stocks, P_{ij}. Mixture sample sizes were fixed at (A) 10 fish/stock (B) 100 fish/stock; learning sample sizes were fixed at 100 fish/stock.

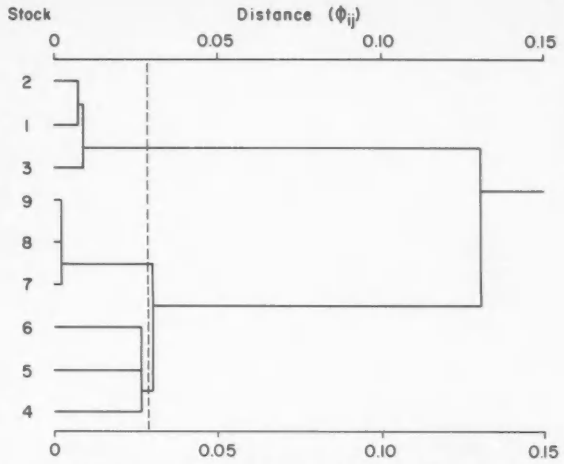


FIG. 11. Similarity dendrogram for the nine hypothetical stocks described in Table 1. The distance measure, ϕ_{ij}, is defined in equation (4). The vertical broken line indicates the level of grouping used to compare the "allocate-sum" and "pool-allocate" clustering procedures.

only results obtained with pooled number of stocks per mixture and learning sample sizes of 100 fish/stock. By doing this we have reduced a six-dimensional problem to a four-dimensional one to facilitate the interpretation of interactions between the three most important independent variables — the number of fish per stock in the mixture, the number of loci, and the separation among stocks — and the resultant magnitude of the model error.

The joint effects of number of loci, separation of stocks, and number of fish per stock in the mixture can be seen in Fig. 9. Note that without adequate separation even large mixture samples cannot be resolved accurately. The effect of reducing the number of loci is apparent by comparing Fig. 9A and 9B. Although the general shape of the surface is quite similar for the two plots, both the magnitude and the slope of the $\bar{\theta}$ surface are greater for the case with fewer loci. The slight increase in error observed in Fig. 9 at a separation of 0.05 and mixture size of 100 fish/stock as compared with the error at 50 fish/stock was unexpected and is attributed to randomness in the simulations. Further analysis revealed that many more Monte Carlo simulations (>200) would be required to evaluate the model's performance more precisely.

We next examined the interaction of stock separation and number of loci with sampling variability controlled by fixing contributions to the mixture (Fig. 10). In these simulations, the lowest error was obtained at the joint maximum values of separation and number of loci and the highest error at the joint minimum. As expected, model performance at low separation with few loci was much improved when "noise" due to sampling variability was reduced by the larger mixture contributions per stock (compare Fig. 10A and 10B). Here, as with the example in Fig. 9, the shape of the $\bar{\theta}$ surface is similar for the two plots, but the magnitude and slope are accentuated for the plot of small mixture size.

Clustering Procedures to Reduce Complexity of Problems

Two approaches to reducing the complexity of mixture problems — the pool-allocate and the allocate-sum procedures (see Methods) — were evaluated using three different mixtures. Composition of the mixture varied from equal contributions by all nine stocks, to randomly selected contributions, to very uneven contributions by only two of the nine stocks (Table 2). Both clustering procedures were based on the similarity dendrogram in Fig. 11 and improved the accuracy of estimates in all cases. In general, the pool-allocate clustering procedure provided more precise, but more biased, estimates than the allocate-sum procedure. It is noteworthy that *all* nonzero estimates using the pool-allocate procedure were significantly biased ($p < 0.05$), due largely to the very low variances of the estimates. These estimates were especially biased for the mixture comprising only two of the nine possible stocks. For this case, mean estimates from the allocate-sum procedure were in error by about 14% whereas mean estimates from the pool-allocate procedure erred by about 34%. The pool-allocate procedure deteriorates under these extreme conditions because the component stocks in the composite learning samples are weighted equally, not weighted by their actual contributions to the mixture. Thus, the composite learning samples are not representative of the corresponding composite sample in the mixture. Even where the composite samples were correctly weighted (i.e. the equal mixture type), the pool-allocate procedure produced a more biased distribution of estimates with lower variance.

Application to Real Problems: An Example

The results from experiments with hypothetical data provide guidelines for approaching more complex, practical problems. In recent years, at least 52 stocks of sockeye salmon in Southeast Alaska and British Columbia have been examined for genetic (electrophoretic) and parasitic characteristics that might be useful for stock identification. The similarity dendrogram in Fig. 12 was constructed using six such characters: the prevalence of a protozoan brain parasite (*Myxobolus neurobius*) and genotypic frequencies at five loci (phosphoglucomutase-1,-2, lactate dehydrogenase-4, and isocitrate dehydrogenase-3,-4) (Bailey 1984; Utter et al. 1984; Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, unpubl. data). Equation (4) was used to determine distances between stocks except that the P_{il} were defined as phenotypic frequencies rather than allelic frequencies in order to include nongenetic traits.

Three mixtures of approximately 500 fish were simulated with the following compositions: (1) equal contributions (1.9% or 10 fish) from each of 52 stocks; (2) 60% (300 fish) from McDonald Lake and 20% (100 fish) from both Meziadin and Little Tatsamenie lakes; and (3) 60% (300 fish) from Babine Lake and 10% (50 fish) from each of Chilko, Tahltan, Bonilla, and Hugh Smith lakes. Each mixture was generated and model estimates obtained 100 times with sampling variability introduced independently to phenotypic frequencies in both the mixture and learning samples. These estimates were summed over stocks that grouped together to the left of the vertical broken lines in Fig. 12 (i.e. the allocate-sum procedure was used). Each vertical broken line defines a different level of stock grouping. Means and variances for the estimated contribution by each stock grouping were computed for each level of grouping; mean angular error was also calculated, since the true mixture composition was known.

The trade-off between level of grouping and the accuracy of the estimates is illustrated in Fig. 13. Mean angular error is

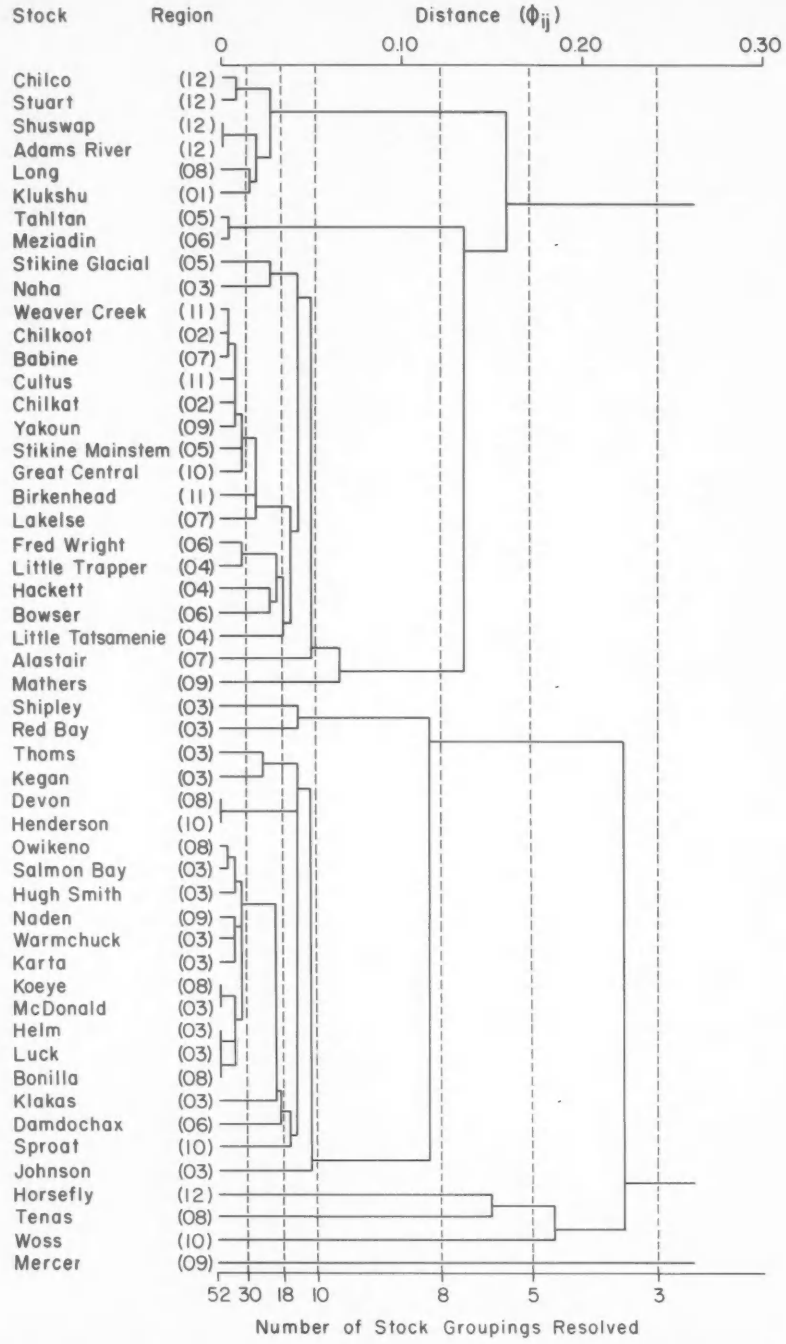


FIG. 12. Similarity dendrogram for 52 sockeye salmon stocks in British Columbia and southeast Alaska based on variation in genotypic frequencies at five loci and the prevalence of a brain parasite. The dendrogram was derived following the same procedures used in Fig. 11; vertical broken lines indicate the six levels of grouping evaluated in Fig. 13. The regions are Alsek River (01), Lynn Canal (02), Southeast Alaska coast (03), Taku River (04), Stikine River (05), Nass River (06), Skeena River (07), north and central British Columbia coast (08), Queen Charlotte Is. (09), Vancouver Is. (10), lower Fraser River (11), and upper Fraser River (12).

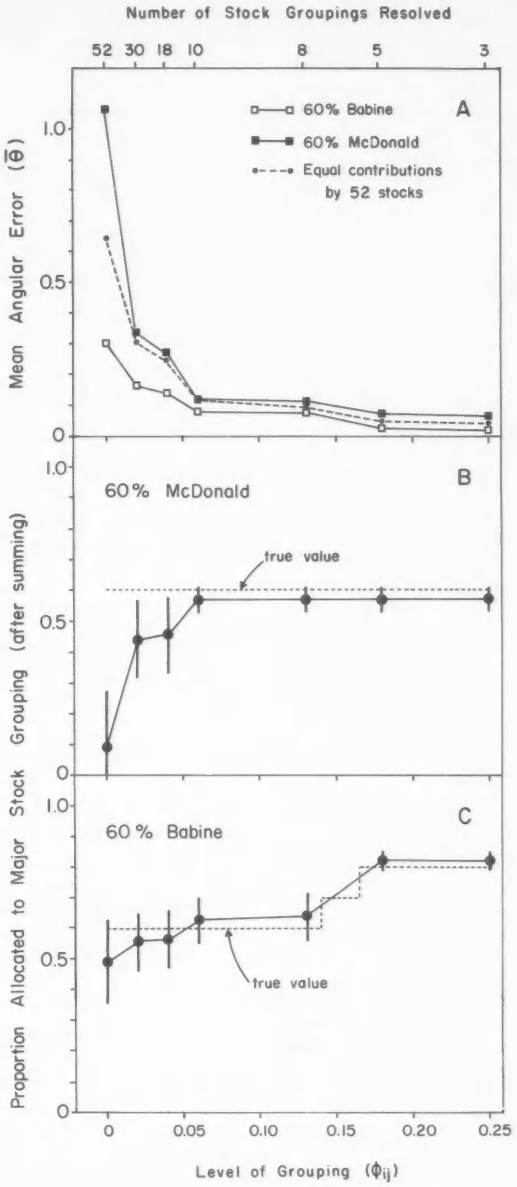


FIG. 13. Results from simulations with mixtures generated from real data for the 52 sockeye salmon stocks presented in Fig. 12. (A) Relationship between level of grouping, ϕ_{ij}, and the mean angular deviation, $\bar{\theta}$, of the estimated mixture composition from the true composition for three different mixtures of approximately 500 fish. Mixture 1 comprised 60% McDonald, 20% Tahltan, and 20% Little Tatsamenie; mixture 2 comprised 60% Babine, 10% Chilco, 10% Meziadin, 10% Bonilla, and 10% Hugh Smith; mixture 3 comprised equal proportions (1.9%) of each stock. (B and C) Relationship between level of grouping and bias and precision for the principal stock groupings in mixtures 1 and 2, respectively. The horizontal broken lines indicate the true proportion; in Fig. 13C, the true proportion changes from 60 to 80% as Meziadin and Chilco are grouped together with Babine for $0.10 < \phi_{ij} < 0.20$.

plotted against level of grouping in Fig. 13A; means with approximate 95% confidence intervals for the estimates (i.e. ± 1.98 SD) are also plotted in Fig. 13B and 13C for the principal contributing stocks with reference to the true values. As expected, the mean estimates converge to the true values, and precision increases, as stocks are clustered into larger groupings. The improvement in accuracy is especially noticeable in Fig. 13B because McDonald Lake falls into a cluster with many very similar stocks.

Discussion

The performance of the mixture model was most affected by three independent variables: the number of discriminating traits used, the amount of separation among stocks, and the number of fish per stock in the samples. The first two variables determine the potential amount of information available for estimating mixture composition; the latter variable determines the level of sampling error on genotypic frequencies, particularly in the mixture, and hence, the amount of information relative to sampling noise that is actually available to the model. At low separation and with few discriminating traits, the model's performance was very sensitive to the effect of sampling error even at realistic sample sizes (>50 fish/stock). Learning sample size had very little effect on model performance for realistic samples >50 fish/stock in these simulations. It is worth emphasizing that the performance of the model was not much affected by the number of stocks per se, but by the number of fish contributed to the mixture by each stock. This implies, of course, that a larger sample size is required to maintain an adequate sample per stock as the number of contributing stocks increases. In practice, sample size requirements must be based on knowledge of the expected number of contributing stocks. Ultimately, the limit to the number of stocks that can be resolved is determined by the number of unique phenotypic combinations (Pella and Milner 1987), or by constraints on computing speed and memory.

The required sample size for both mixture and learning sample sizes will also depend on the number of observable states (i.e. phenotypes) per trait. The frequency of each phenotype in a population is a parameter to be estimated from the corresponding learning sample. Thus, a larger sample size is required to estimate a larger number of parameters (e.g. Altham 1984). It is true that a single trait with many observable phenotypes could permit, in principle, the resolution of a larger number of different stocks than would be possible with a single trait with very few observable phenotypes. However, the effect of sampling variability on the observed phenotypic frequencies will be much more pronounced for the trait with many observable phenotypes; consequently, proportionately larger sample sizes would be required to utilize a comparable level of separation among stocks. This sampling variability will be a special problem for genotypic frequencies for loci within $n \geq 3$ alleles ($n + n!/(n-2)!2!$ genotypes) compared with loci with only two alleles (three genotypes), since many or all of the potentially occurring genotypes are likely to be observed in most stocks. This sampling problem can be reduced to some extent by "correcting" observed genotypic frequencies in the learning samples to that expected under the corresponding multinomial distribution (i.e. Hardy–Weinberg equilibrium). In effect, this procedure reduces the number of states per trait to the number of alleles. Unfortunately, no such correction is possible for the mixture samples. From the perspective of sampling economy,

it seems preferable to seek a large number of traits, each with relatively few observable phenotypes, provided all traits can be measured for a single fish.

The use of additional loci in our simulation experiments demonstrated the *minimum* improvement in performance that might be expected because all loci were identical, although independent. In real applications, individual traits would be expected to separate some stocks better than others, and each trait would be expected to "key out" different groups of stocks. Consequently, the performance of the model should be greatly improved when traits "acting in different directions" are used collectively.

Stock identification problems too complex to solve directly can be simplified by examining the patterns of similarity among stocks that might be present in the mixture. We recommend the allocate-sum procedure, whereby estimated proportions are summed across stocks with similar attributes, to generate improved estimates of the contributions by a reduced number of stock groupings. This procedure has a number of advantages over the alternative pool-allocate procedure. In simulations with nine hypothetical stocks, estimates obtained with the former method were generally less biased than those obtained using the latter procedure, especially where contributions from stocks within a grouping were not equal. The allocate-sum procedure is also easier to use and evaluate. First, it is easier to maintain a single data file containing all original learning samples than to continually create new pooled learning samples for specific problems. Second, the estimates from a single set of simulations can be summed at various levels of grouping to evaluate the most appropriate compromise between accuracy and stock resolution — the intensive estimation routine need not be repeated using different composite learning samples. It does seem reasonable, however, to pool learning samples for stocks whose observed phenotypic distributions are not significantly different.

The accuracy of estimates obtained with the allocate-sum procedure depends to a large extent on the qualities of the similarity dendrogram used to recognize stock groupings. The distance measure we have used in the example problem (equation (4)) is derived from the genetic distance statistic (Nei 1972) and gave satisfactory results. In these simulations, it proved superior to the likelihood ratio statistic (e.g. the G statistic of Sokal and Rohlf 1981) which is a function of both learning sample size and the genotypic frequencies. However, it is apparent from Fig. 2 that the genetic distance measure has one undesirable attribute — three stocks separated by a fixed genetic distance were more easily resolved by the mixture model as the two allelic frequencies (for a single locus) became less similar (i.e. one allele became rare). An ideal distance measure should accurately reflect the difficulty of resolving two stocks with the mixture model by taking into account the number of traits, the number of phenotypes per trait, or the evenness of the phenotypic frequencies.

Millar (1987) has suggested using the covariance matrix of stock composition estimates to guide decisions about which estimates to sum, or which stocks to pool. Ranking correlations, and summing estimates for stocks with the most negative values first, might result in more rapid convergence to the correct estimates for larger stock groupings. Although this procedure could possibly provide superior summing rules for any particular mixture problem, it will provide less perspective on all possible mixture problems. Dendrograms based on distances from equation (4) summarize the information in the learning samples and provide a convenient way to judge stock resolution potential before conducting the intensive estimation routine. Thus, dendrograms can be computed very quickly using a variety of different traits to determine which combination of traits will be most effective at differentiating stocks of concern, for any conceivable mixture problem. Once this choice of traits has been made, and the estimation procedure has been completed for a particular mixture sample, it may be desirable to derive more efficient summing rules from the corresponding covariance matrix of the stock composition estimates.

It is unwise to sum estimated proportions (or pool learning samples) for stocks grouped together for the convenience of management or allocation objectives where these artificial groupings do not correspond with groupings based on stock similarity. This is equivalent to using an inappropriate distance measurement to define stock groupings. In difficult problems where stock separation is inadequate or sample sizes are too small, estimates for individual stocks are usually badly biased. This bias can be minimized or eliminated by judicious grouping of stocks as already described. No such improvement in accuracy can be expected by summing estimates within incorrect stock groupings; in fact, accuracy would deteriorate if stocks with positively correlated estimates were grouped together.

Where stock groupings based on management objectives conflict with natural groupings based on similarity, additional discriminating traits must be found to achieve the desired stock resolution while maintaining an acceptable level of accuracy. However, one virtue of the allocate-sum procedure is that certain potentially occurring stocks can be discounted later, given additional information, without affecting the original analysis. For example, it is often possible to judge which stocks within problem clusters are unlikely to have been present in the mixture based on run timing, knowledge of migration routes, distance from the fishery, and magnitude of escapement. Such a posteriori judgements can be made explicit by managers once the mixture model can no longer guide their decisions. For this reason, we feel it is usually preferable to sacrifice stock resolution in favour of increased accuracy. Graphical analyses of this trade-off (e.g. Fig. 13) can greatly facilitate the task of choosing an effective compromise.

The maximum-likelihood mixture model offers much promise for solving a wide variety of difficult stock-identification problems. Our sensitivity analysis demonstrates that the mixture model behaves in a manner consistent with intuitive expectations, and it is reasonable to expect that the results of these simulations can be extrapolated to real problems. However, we advise new users to proceed cautiously, since specific results may depend on assumptions in our experimental design. In particular, it is very important to investigate the characteristics of the minimization algorithm used to estimate the model parameters; assumptions about initial values and cut-off criteria may have a critical influence on the final estimates for difficult problems (see also Pella and Milner 1987). Estimates will always be generated and it is not always easy to recognize nonsensical results. Thus, the analyst must learn to appreciate the capabilities of both the mixture model and the minimization algorithm before using the resulting estimates for fisheries management decisions where error could be costly.

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computations. Drs. Brian Riddell and Leo Margolis kindly provided unpublished electrophoretic and parasite data for sockeye populations used in the example problem. Discussions with Drs. Brian Riddell, Jerry Pella, Paul Sampson, Jon Schnute, Terry Beacham, and Russell Millar helped to focus our efforts and were much appreciated. We are also grateful to Drs. Pella and Millar for their careful review of the manuscript.

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Appendix

There are many statistical procedures for estimating the composition of mixtures of statistical distributions (e.g. Smith and Makov 1978). Two methods commonly used for estimating the composition of mixed-stock fisheries are the maximum-likelihood estimation for a mixture problem with learning samples procedure as described by Milner et al. (1981, 1983) and Fournier et al. (1984) and the classification-with-correction approach initiated by Worlund and Fredin (1962) and extended by Cook and Lord (1978) and Pella and Robertson (1979). In this appendix, we compare the two approaches in the parametric case where the observed characteristics are represented by a random vector whose probability mass function has a known functional form depending on a set of unknown parameters. We shall consider the case of discrete random variables, but the case of continuous random variables is similar. It is also possible to compare the two methods for the case of nonparametric density estimation considered in Cook and Lord (1978). The case we consider here is the simplest and it serves to illustrate the main point — that the classification-with-correction procedure involves a grouping of the observations which can lead to a loss of information. It is therefore intrinsically inferior to the conditional likelihood method.

Assume that there are K stocks indexed by j which could occur in the mixture. From each stock j, an independent random sample of fish is taken and for each fish r, a vector of characteristics $\boldsymbol{X}_{jr}$ is observed. It is assumed that for each stock the vector of observed characteristics for a fish from that stock is a random vector with a probability mass function $f_j(\boldsymbol{X}; \boldsymbol{A}_j)$ which depends on the unknown parameters $\boldsymbol{A}_j$. In addition, there is also a random sample of fish indexed by s which is taken from the mixture, and $\boldsymbol{Y}_s$ is the vector of characteristics of the sth fish taken from the mixture.

Let $p_j, j = 1, \ldots, K$, be the proportion of the mixture which is composed of the jth stock. The maximum-likelihood estimates for the $\boldsymbol{A}_j$ and p_j are found by maximizing the likelihood function, i.e. by finding the solution to the problem

$$\max_{p_j, \boldsymbol{A}_j} \left\{ \sum_j \sum_r \log_e \left(f_j(\boldsymbol{X}_{jr}; \boldsymbol{A}_j) \right) + \sum_s \log_e \left(\sum_j p_j f_j(\boldsymbol{Y}_s; \boldsymbol{A}_j) \right) \right\} \tag{A.1}$$

subject to the constraints

$$p_j \geq 0 \text{ and } \sum_j p_j = 1.$$

Rather than dealing with the somewhat difficult maximization problem (A.1), Fournier et al. (1984) first found the values $\hat{\boldsymbol{A}}_j$ which solve the problem

$$\max_{\boldsymbol{A}_j} \left\{ \sum_j \sum_r \log_e \left(f_j(\boldsymbol{X}_{jr}; \boldsymbol{A}_j) \right) \right\}. \tag{A.2}$$

These are the maximum-likelihood estimates for the $\boldsymbol{A}_j$ given the learning samples alone. They then estimate the p_j by finding $\hat{p}_j$ which solve the problem

$$\max_{p_j} \left\{ \sum_s \log \left(\sum_j p_j f_j(\boldsymbol{Y}_s; \hat{\boldsymbol{A}}_j) \right) \right\}. \tag{A.3}$$

The $\hat{p}_j$ are referred to as conditional likelihood estimates because they are the maximum-likelihood estimates for the p_j based on the condition that the $\hat{\boldsymbol{A}}_j$ are the correct values for these parameters.

Consider the classification-with-correction procedure. The first part of the procedure is the classification process, that is, the development of a rule which will assign each individual fish to a stock. The second part of the procedure is the estimation of the probability of misclassifying individuals from each stock to another stock. To carry out this procedure, the known-origin samples must be split into two parts, called the learning and test samples, so that the learning sample can be used to formulate the decision rule, while the test sample is used to evaluate the probability of misclassification when using this rule.

Assume that the correct values of the parameter vectors are known. As Pella and Robertson (1979, p. 387) pointed out, "among rules, those with the lowest error rates in assignments provide the most accurate assessments." If a fish has a vector of characteristics X, the classification rule with the lowest error is to assign the fish to group k where k satisfies the condition

$$f_k(X; A_k) > f_j(X; A_j)$$

for all other groups j. This classification rule will be referred to as the dominant likelihood rule. When the probability density functions are known, the dominant likelihood rule is the classification rule with the lowest error rate.

For each, j, let Ω_j be defined by

$$\Omega_j = \{X: f_j(X; A_j) > f_k(X; A_k) \text{ for } k = 1, \ldots, K\}$$

so that Ω_j is the set of all vectors of observations for which the fish will be classified as belonging to group j by the dominant likelihood rule. Let $C_{jk}(A_j)$ be defined by

$$\text{(A.4)} \quad C_{jk}(A_j) = \sum_{X \in \Omega_k} f_j(X; A_j)$$

so that C_{jk} is the probability that a fish from stock j will have a vector of characteristics lying in Ω_k. Let n_k denote the number of fish in the random sample from the mixture whose vector of characteristics lies in Ω_k. The maximum-likelihood estimates for the p_j given the n_k is found solving the problem

$$\text{(A.5)} \quad \max_{p_j} \left\{ \sum_k n_k \log_e \left(\sum_j p_j C_{jk}(A_j) \right) \right\}.$$

The estimates for the p_j are, in general, inferior to the conditional likelihood estimates because information has been lost by grouping the observations over the sets Ω_j.

Let q_k be the proportion of the observations from the mixture which lie in Ω_k, i.e.

$$q_k = n_k \Big/ \sum_j n_j.$$

Proposition 1

If there exists $\tilde{p}_j$ satisfying the conditions

$$\text{(A.6)} \quad q_k = \sum_j \tilde{p}_j C_{jk}(A_j)$$

where

$$\text{(A.7)} \quad p_j \geq 0 \text{ and } \sum_j \tilde{p}_j = 1,$$

then the $\tilde{p}_j$ are the solution to the maximization problem (A.5).

Proof

This follows immediately by noting that the quantities q_k are the global solution to the maximization problem

$$\max_{q_k} \left\{ \sum_k n_k \log_e (q_k) \right\}$$

over all quantities q_k satisfying the conditions

$$q_k \geq 0 \text{ and } \sum_j q_j = 1.$$

Of course, $\tilde{p}_j$ satisfying (A.6) and (A.7) may not always exist.

Assume that the matrix $C_{jk}(A_j)$ is invertible and let $D_{kj}(A_j)$ denote the elements of its inverse.

Proposition 2

Let

$$\tilde{p}_j = \sum_k D_{kj}(A_j)\, q_k.$$

Then, if the $\tilde{p}_j$ satisfy the constraints (A.7), they are the maximum-likelihood estimates solving the maximization (A.5) and they are also the classification-with-correction estimates of Pella and Robertson (1979).

Proof

It follows from proposition 1 that the $\tilde{p}_j$ will solve the maximization (A.5). Note that the $C_{jk}(A_j)$ are the probabilities that a fish from stock j will be classified as belonging to stock k under the dominant likelihood rule and that the q_k are the proportion of the fish in the mixture sample classified as belonging to stock k. It follows that the $\tilde{p}_j$ are identical to the classification-with-correction estimates of Pella and Robertson (1979) in the case where the parameters A_j are known. To the extent that grouping the observations over the regions Ω_j can lead to a loss of information, the estimates derived from the classification-with-correction procedure will be inferior to the conditional likelihood estimates.

In the parametric case, the classification-with-correction procedure is also inefficient because the known origin samples must be broken up so that part of them is used to formulate the classification rule, while the rest is used to estimate the probability of misclassification. While this problem can be alleviated by jackknifing (Cook 1982), the fact remains that this need to separate the samples is really just an artifact of the two-stage classification-with-correction procedure. The probability mass function is forced to serve two purposes: to define the decision regions Ω_j and to determine the probability of misclassification. With the conditional likelihood approach, this artificial dichotomy is removed so that the data can be used in the most effective manner.

A Simulation Example

As an example, we have simulated the analysis for a two-stock mixture problem. There is one discrete character which can take on four states. Two sets of simulations were performed, one set for a mixture consisting of 50% from each stock and a second set consisting of a mixture of 30% stock 1 fish and 70% stock 2 fish. The probability distributions for the characters are as follows:

	State			
Stock	I	II	III	IV
1	0.10	0.50	0.39	0.01
2	0.01	0.39	0.50	0.10

If a member of the mixture is picked and a character observed, it will be classified as belonging to stock 1 if the character lies in state I or II and in stock 2 if the character lies in state III or IV. Thus, Ω_1 consists of states I and II, while Ω_2 consists of

states III and IV. The probability distribution for the grouped observations is given by the following:

	Grouped states	
Stock	Ω_1	Ω_2
1	0.60	0.40
2	0.40	0.60

Notice that the grouping has led to the loss of the large contrast in the probabilities which existed in states I and IV.

For the computer simulations, a learning sample of 200 fish was generated from each of the stocks, and a mixture sample of 400 fish was generated from a mixture consisting of 50% each of the two stocks. This procedure was repeated 200 times for both the grouped and ungrouped data with the following results (for $\hat{p}_1$):

	Mean	SD	Min.	Max.	1st quart.	Median	3rd quart.
Ungrouped	0.50	0.13	0.04	0.82	0.41	0.51	0.59
Grouped	0.51	0.19	0.00	1.00	0.37	0.51	0.63

The same procedure was repeated for a mixture containing 30% stock 1 fish and 70% stock 2 fish. Results for $\hat{p}_1$ were as follows:

	Mean	SD	Min.	Max.	1st quart.	Median	3rd quart.
Ungrouped	0.30	0.12	0.00	0.60	0.21	0.31	0.37
Grouped	0.30	0.18	0.00	0.93	0.16	0.30	0.44

Both sets of simulations demonstrate the loss of information caused by grouping the observations. In both cases the standard deviation of the estimate for p_1 is increased substantially, from 0.13 to 0.19 for the 50%−50% mixture and from 0.12 to 0.18 for the 30%−70% mixture.

Recruitment Dynamics of Spring-Spawning Herring in the Northwest Atlantic

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Recruitment patterns of the seven major spring-spawning herring populations in the Northwest Atlantic were found to be determined largely by annual variations in overwintering temperatures and salinities associated with the Labrador Current, and the patterns were therefore synchronous. Removal of the environmental component of recruitment variability revealed dome-shaped stock–recruit curves with sharply ascending left limbs and relatively high degrees of compensatory density dependence in recruitment as measured by the Ricker parameter, β. The degree of such density dependence was positively correlated with recruitment variability which was itself negatively associated with mean stock size, i.e. large herring stocks tended to have less recruitment variability but lower levels of density dependence in recruitment than smaller stocks. In addition, environmental variability was positively associated with recruitment variability, and for stocks of the same size, the more northern populations of spring-spawning herring, inhabiting a more variable environment, have a higher level of density dependence on recruitment. Simulation studies showed that larger herring stocks could sustain higher levels of fishing mortality than smaller herring stocks, due mainly to reduced recruitment variability. We suggest that the most appropriate fishing strategy for herring is to maintain fishing mortality at a level of $F = 0.20$ with the option of closure when parent stock size falls below the critical level. A surprising finding from our study was the high level of density dependence of recruitment in herring stocks which we attributed to several features: (1) a large egg size and the ability of herring populations to adapt to a wide range of spawning conditions, (2) a density-dependent mortality associated with egg hatching success, and (3) a strong homing tendency ensuring that changes in spawning population sizes are spread evenly throughout the spawning area, thereby optimizing larval production.

Les auteurs ont découvert que les régimes de recrutement chez sept grandes populations de hareng reproducteur de printemps dans l'Atlantique nord-ouest sont en grande partie déterminés par des variations annuelles des températures et des salinités hivernales liées au courant du Labrador; les régimes étaient donc synchrones. L'élimination de la composante environnementale de la variabilité du recrutement révèle des courbes stock–recrue en forme de dôme dont la partie gauche est à pente raide et qui présentent des niveaux relativement élevés de dépendance compensatoire liée à la densité pour ce qui est du recrutement quantifié par le paramètre β de Ricker. Le niveau d'une telle dépendance liée à la densité était en corrélation positive avec la variabilité du recrutement qui lui-même était négativement associé avec la taille moyenne du stock; en d'autres mots, les importants stocks de hareng tendaient à avoir une plus faible variabilité du recrutement et de faibles niveaux de dépendance liée à la densité pour ce qui est du recrutement, par rapport aux petits stocks. De plus, la variabilité environnementale était en association positive avec la variabilité du recrutement, et chez des stocks de même taille, les populations plus septentrionales de hareng reproducteur de printemps peuplant un environnement plus variable montraient un niveau plus élevé de dépendance liée à la densité dans le cas du recrutement. Des études de simulation ont montré que les grands stocks de hareng pouvaient soutenir des taux plus élevés de mortalité par pêche que les petits stocks, surtout à cause d'une variabilité réduite du recrutement. Pour ce qui est de la stratégie la plus appropriée de pêche du hareng, les auteurs proposent de maintenir la mortalité par pêche à $F = 0{,}20$ avec une option de fermeture quand la taille du stock parental tombe au-dessous du niveau critique. Le niveau élevé de dépendance du recrutement des stocks de hareng vis-à-vis de la densité, une découverte étonnante, a été attribué à plusieurs facteurs: 1) une taille élevée de l'oeuf et la capacité d'adaptation des populations de hareng à des fortes variations des conditions de fraie, 2) une mortalité dépendante de la densité et liée au succès de l'éclosion des oeufs et 3) une tendance marquée de retour vers les frayères natales qui assure que les variations chez les populations reproductrices sont uniformément réparties dans toutes les frayères, ce qui optimise la production de larves.

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A common characteristic of nearly all herring fisheries that have collapsed was their abrupt demise without evident symptoms of distress. A classic example of this phenomenon is the Georges Bank herring stock which sustained catches in excess of 140 000 t for the decade 1966–75 and then collapsed to less than 2000 t by 1977 (Anthony and Fogarty 1985). Retrospective analyses showed that recruitment levels plummeted from near record levels for the 1970 year-class to virtually nil for the 1971 and subsequent year-classes (Anthony and Fogarty 1985), yet current stock

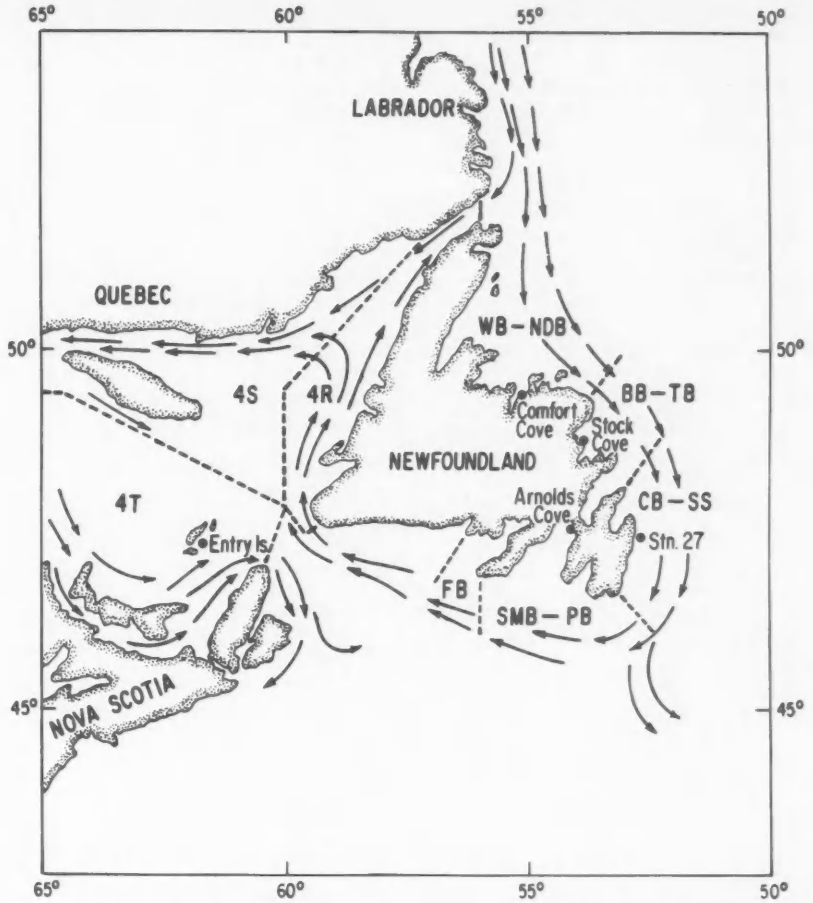

FIG. 1. Map showing stock areas mentioned in the text. Arrows depict the general pattern of the Labrador Current. (Redrawn from Trites 1982.) Data sources: White Bay – Notre Dame Bay (WB-NDB), Bonavista Bay – Trinity Bay (BB-TB), Conception Bay – Southern Shore (CB-SS) from Wheeler et al. (1985); St. Mary's Bay – Placentia Bay (SMB-PB) from Wheeler and Dalley (1985) and Winters et al. (1986); Fortune Bay (FB) from Wheeler and Dalley (1985); northern Gulf (4R) from Tremblay et al. (1983) and McQuinn and Cleary (1985); southern Gulf (4T) from Cleary (1984) and Winters (1976).

assessments failed to detect this trend (Anthony and Waring 1980). Such rapid recruitment failures suggest a stock–recruit curve with a very steep left-hand limb and a relatively low threshold (i.e. critical) spawning biomass. Such a stock–recruit relationship is probably associated with an over-abundant egg production, and therefore, recruitment success will be largely determined by density-independent factors. For a wide variety of pelagic fish stocks such as capelin (Leggett et al. 1984), Atlantic menhaden (Schaaf 1980), and Pacific herring (Stocker et al. 1985), environmental factors are believed to be the main regulators of year-class success. These environmental signals are effective over wide areas (Sutcliffe et al. 1976; Koslow 1984), and therefore, synchrony in recruitment success would be expected of stocks whose environments are dominated by common oceanographic events.

The marine climate of Newfoundland (Fig. 1) is greatly influenced by annual variations in the physical parameters of the Labrador Current (Huntsman et al. 1954; Trites 1982; Sutcliffe et al. 1983). In addition, fluctuations in these parameters in adjacent areas such as the southern Gulf of St. Lawrence are both influenced by and synchronous with variations in the Labrador Current. For example, Gulf of St. Lawrence water temperatures are positively correlated with those of the Labrador Current (Moores and Winters 1982), and there is also a significant relationship between St. Lawrence River discharge and Labrador Current salinities (Koslow 1984). Substantial populations of spring-spawning herring exist in all of these areas (Pinhorn 1976), and temperature variability has been shown to be the main factor associated with variations in the annual growth of several of these stocks (Moores and Winters 1982; Winters et al. 1986). In this paper, recruitment patterns of the seven major spring-spawning herring stocks influenced by the Labrador Current are examined. We suggest that their recruitment successes are determined mainly by

annual variations in the physical parameters of the Labrador Current and are therefore synchronous. In addition, we develop empirical models of recruitment which remove the variability due to environmental factors and reveal the characteristics of the underlying stock–recruit relationships. We posit that these general stock–recruit curves will be dome shaped and that interstock variations in the density-dependent parameter will be primarily a function of interstock differences in mean stock size as mediated through recruitment variability. Simulation studies involving a stochastic environmentally dependent stock–recruit model assess the impact of recruitment variability on fishery yields at varying fishing mortality rates.

Materials and Methods

We have restricted our analyses to those major spring-spawning populations of herring in the Northwest Atlantic for which stock assessment data are available and whose stock boundaries have been adequately defined by a variety of stock discrimination techniques. The data sources for population and recruitment estimates are given in Fig. 1, and the reader is referred to Parsons (1973), Moores and Winters (1984), Wheeler and Winters (1984a), and Winters and Beckett (1978) for a description of the basis for the definition of stock units.

Age-specific fecundities (F_t) were calculated from annual weight-at-age data and from a combined fecundity–weight relationship for spring-spawning herring stocks in Placentia Bay, Fortune Bay, and southwest Newfoundland (i.e. 4T herring) as described by Hodder (1972). Fecundity–weight data from these stocks were combined on the basis that Hodder (1972) reported no significant differences in the fecundity–weight relationship in samples of herring from these areas. Age-specific maturation rates (M_t) were derived for each stock from commercial sampling data collected during the first half of the year from all gears except gill nets. In the absence of annual maturation rate data for all stocks and years, mean maturation rates were calculated for each stock from its historical data base covering the time period involved in these analyses. Population fecundity in year t (E_t) was then calculated from midyear population numbers (N_t) according to the following algorithm (Winters et al. 1985):

$$E_t = \Sigma\, M_t \cdot F_t \cdot N_{t/2}.$$

Temperature and salinity data used in these analyses refer to the 0–20 m surface layer and are based on data recorded at Station 27 (47°31′50″N, 52°35′10″W) off St. John's, Newfoundland (Fig. 1) (Akenhead 1983). This monitoring site has one of the longest uninterrupted time series of hydrographic data in the Northwest Atlantic and provides a reliable index of the Labrador Current as a measure of general environmental trends in the Newfoundland area (Sutcliffe et al. 1983). The surface layer was chosen because its physical parameters closely approximate those under which egg development and early larval development of spring-spawning herring occur (Winters et al. 1985) and because it may reveal annual variations in the nutrient mixing process of the Labrador Current (Sutcliffe et al. 1983). Temperature (T) and salinity (S) data were gathered for the period 6 mo prior to spawning (T_1, S_1), i.e. overwintering/prespawning period (November–April), and 6 mo following spawning (T_2, S_2), i.e. summer/postspawning period (May–October), because these periods were considered to have biological significance (Winters et al. 1985, 1986). For example, winter water temperatures have been shown to affect the year-class strength of spring-spawning herring in the Baltic (Rannak 1971). In addition, high salinities and warm overwintering temperatures can be taken as indicative of upwelling intensity (Walford 1946), and the importance of low-salinity water as an inhibitor of nutrient mixing of the Labrador Current has been demonstrated by Sutcliffe et al. (1983). Furthermore, such overwintering environmental conditions have been shown to control the development and spawning time of zooplankton (Mankowski 1966), the reproductive products of which form a predominant component of the prey of herring larvae (Bainbridge and Forsyth 1971). Finally, it is now generally accepted that year-class strength in many fish stocks is fixed by the end of the larval period (Leggett et al. 1984), which is October in the case of spring-spawning herring, and both temperature and salinity conditions during and immediately after spawning have been shown to affect both hatching success and survival of herring larvae (Taylor 1971; Holliday and Blaxter 1960).

Comparable site-specific temperature data at a standard depth of 9–10 m were only available for five of seven herring stocks considered in this study (Dobson and Petrie 1984) and data on file at the Northwest Atlantic Fisheries Centre (Dr. G. P. Ennis, pers. comm.).

Estimates of recruitment, R_t, (at age-group 2) are available from published stock assessment studies (Fig. 1, legend) for all stocks for the 1964–76 year-classes. More recent year-classes (1977 and later) were excluded from the analysis because for most stocks such estimates would be sensitive to the terminal year input parameters of cohort analyses, particularly the fully recruited fishing mortality rate. Recruitment estimates for the 1963 year-class and egg production levels (E_t) for the years 1963–65 were derived from a back-calculation of the 1966 population vector, assuming $M = 0.20$. Fishing mortality rates were considered to be negligible for all stocks for the 1963–65 period, based on the 1966 level of fishing mortality and the low catches recorded for all stocks for the years 1963–65.

Recruitment data (R_t) were transformed to natural logarithms because herring recruitment follows a lognormal pattern (Hennemuth et al. 1980). Since herring stocks often exhibit long-term trends in recruitment, significant correlation coefficients were tested for spurious time-series correlations by detrending the data and taking first-order differences (Box and Jenkins 1970):

$$(1)\quad \log_e R_0 = \log_e R_t - \log R_{t-1}$$
$$E_0 = E_t - E_{t-1}$$
$$T_0 = T_t - T_{t-1}$$
$$S_0 = S_t - S_{t-1}.$$

Recruitment variability was evaluated for each stock using a multiple stepwise regression model with T_1, T_2, S_1, and S_2 as optional independent variables. An independent variable was accepted into the model if its inclusion resulted in a significant increase in the R^2 level and a significant decrease in the mean-squared error, based on the partial F test.

Stock–recruit curves were derived using the environmentally dependent Ricker stock–recruit model (Stocker et al. 1985), since such analyses allow the underlying stock–recruit relationship to be described unmodified by the variability due to environmental factors. The environmental Ricker model was transformed to linear function by taking natural logarithms:

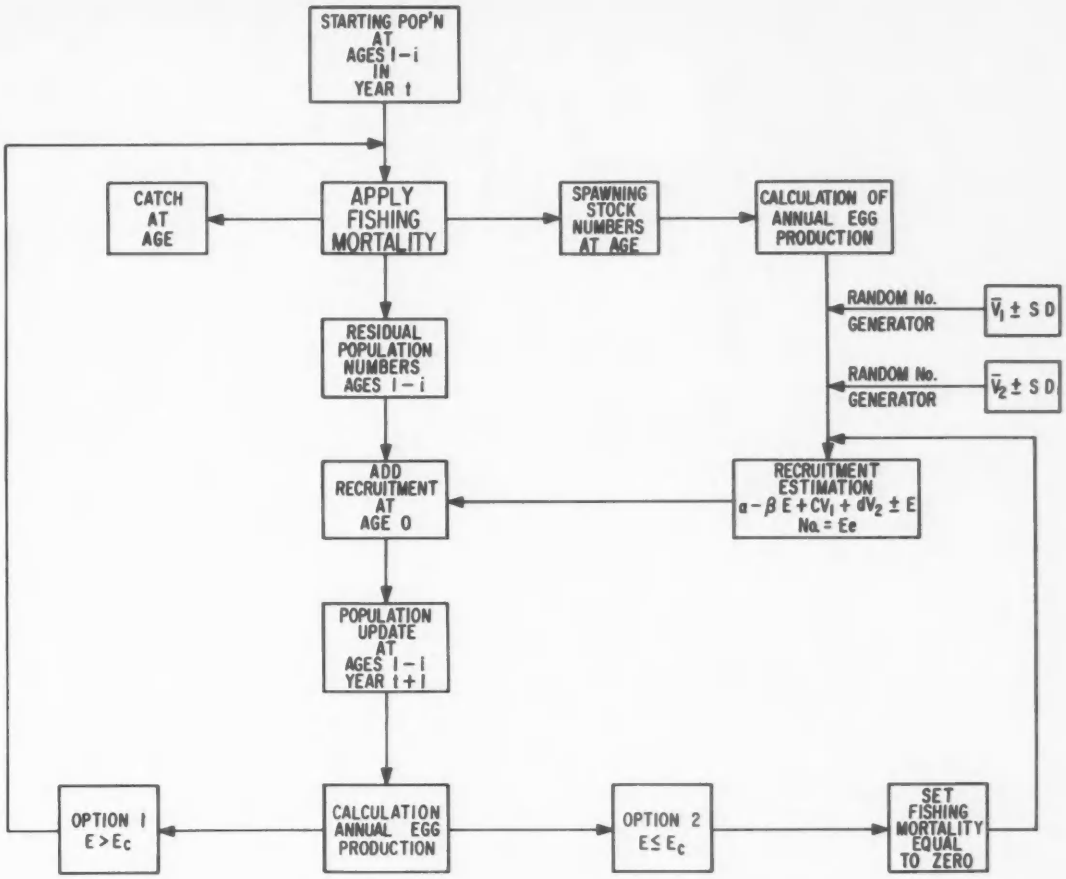
STARTING POP'N AT AGES I–i IN YEAR t
CATCH AT AGE
APPLY FISHING MORTALITY
SPAWNING STOCK NUMBERS AT AGE
CALCULATION OF ANNUAL EGG PRODUCTION
RANDOM No. GENERATOR
$\bar{V}_1 \pm$ S D
RANDOM No. GENERATOR
$\bar{V}_2 \pm$ S D
RESIDUAL POPULATION NUMBERS AGES I–i
ADD RECRUITMENT AT AGE 0
RECRUITMENT ESTIMATION
$\alpha - \beta E + CV_1 + dV_2 \pm E$
No. = Ee
POPULATION UPDATE AT AGES I–i YEAR t+1
OPTION 1 E>Ec
CALCULATION ANNUAL EGG PRODUCTION
OPTION 2 E≤Ec
SET FISHING MORTALITY EQUAL TO ZERO

FIG. 2. Schematic diagram of the simulation model of the impact of recruitment variability and a stochastic stock–recruit function on yield dynamics of Atlantic herring.

$$(2)\quad \log(R/E) = \alpha - \beta E + CV_1 + dV_2 + \epsilon$$

where R = estimated recruitment, E = annual egg production, V_1 and V_2 = key environmental variables, β and α = Ricker stock-recruit parameters, and ϵ = normally distributed random variables with mean σ and variance σ^2.

Retransformation of (2) gives the following recruitment model:

$$(3)\quad R = Ee^{(d\ -\ \beta E\ +\ CV_1\ +\ dV_2\ +\ \epsilon)}.$$

The interaction between recruitment variability, stock–recruit dynamics, and fishing mortality was assessed using a stochastic simulation model whose central component was the environmentally dependent recruitment algorithm given in expression (3). Data requirements for each stock include an initial population structure, critical spawning stock size (E_c), age-specific weights, fecundity-at-age (adjusted for maturation rates and sex ratio), partial recruitment rate (constant for all stocks), maximum life span, natural mortality rate (M), and statistical distributions (mean, standard deviation) of the various environmental variables included in the recruitment model. Where possible, data input are based on empirical estimates derived for each stock. It is assumed that density-dependent changes in growth and maturity are negligible for these herring stocks (Moores and Winters 1982), as they are, indeed, for most herring stocks (Smith 1985; Doubleday 1985). A schematic description of the model is shown in Fig. 2.

The simulation model is stochastic in the sense that recruitment estimation includes the parameter ϵ (i.e. the multiplicative lognormal error of the Ricker model) as well as depending on a random number generator to select annual estimates of the various environmental variables from their respective statistical distributions. Essentially the model subjects an initial population structure (chosen randomly from trial runs) to a defined level of fishing mortality (F) which is assumed to occur evenly throughout the year. Because these herring populations are spring spawners, midyear population estimates (ages 1 to the defined life span) are then derived and then an estimate of annual egg production is calculated. This population fecundity estimate is then inserted into the particular environmentally dependent recruitment model along with estimates of the selected environmental variables as determined from a random selection within their respective statistical distributions. This procedure results in a stochastic estimate of

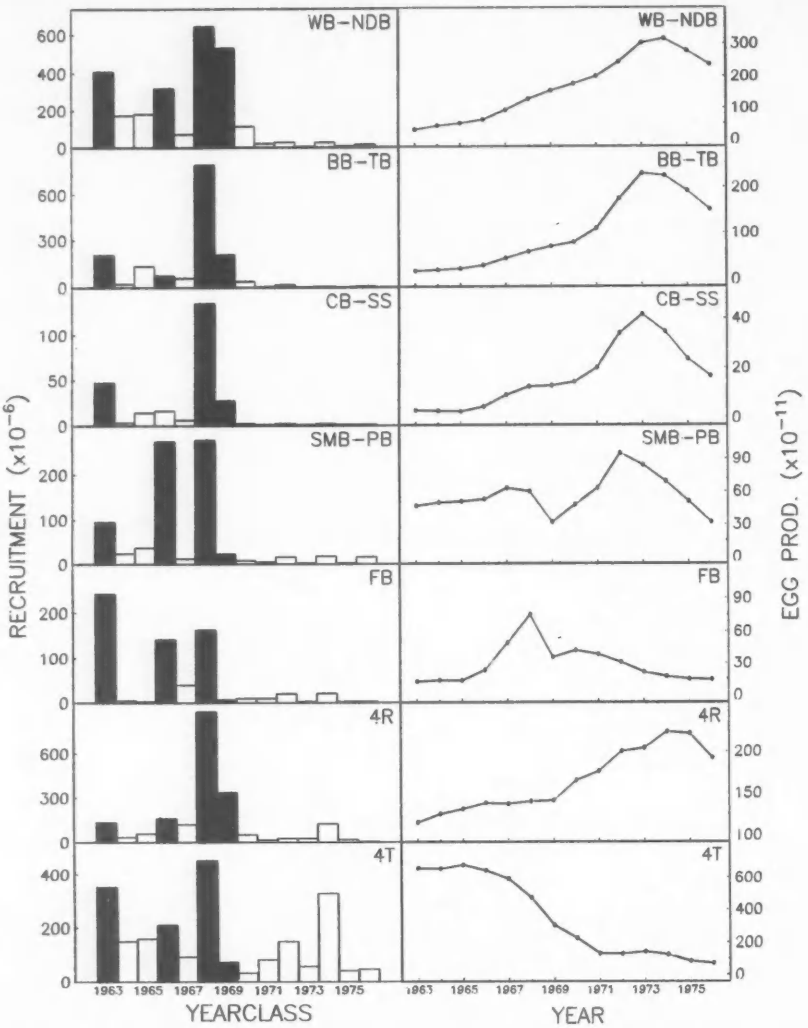

FIG. 3. Time series of recruitment (log-transformed numbers at age 2) and egg production estimates of the various spring-spawning herring stocks for the 1963–76 year-classes. The shaded blocks indicate year-classes which are dominant in most stocks.

recruitment (at age 0) which is then added to the initial age structure and projected forward to the next year according to the age-specific mortality rates ($Z_t = F_t + M$). This provides an updated age structure and the above sequential operations are then repeated according to two options of fishing strategy: (1) unrestricted fishing in which the specified level of fishing mortality is maintained regardless of spawning stock size and (2) fishing mortality reduced to zero when egg production goes below the critical spawning stock size (E_c). The simulation was run for 75 yr for each stock and the results were averaged from a composite of 50 replicates. Summary outputs include recruitment estimates (±SD), catch and population biomass (±SD), egg production estimates (±SD), and the mean and standard deviation of the various environmental variables as chosen randomly from their distributions.

Results

Recruitment Synchrony

The time series of recruitment and egg production estimates for the various stocks and the selected environmental parameters are shown in Fig. 3 and 4, respectively. In all stocks but 4T, recruitment has been relatively low in the 1970's compared with the 1960's. In general, the 1963 and 1968 year-classes were strong in most areas, with the 1966 year-class being strong in four of the seven stocks examined. The 1974 year-class showed the greatest interstock differences in relative strength, being weak in all areas except 4R and 4T. In general, however, a pattern of consistency in recruitment variability is evident and this is confirmed by correlation analysis of the

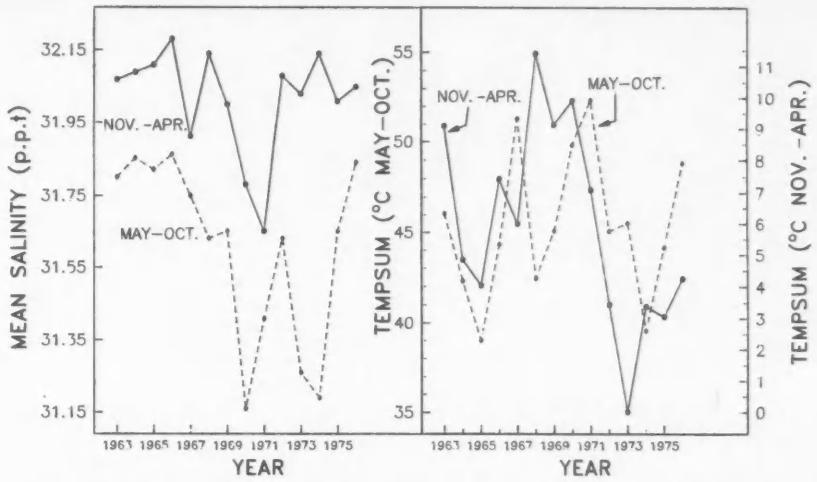

FIG. 4. Time series of salinity (S_1 = sum of monthly means for November–April; S_2 = sum of monthly means for May–October) and temperature (T_1 = sum of monthly means for November–April; T_2 = sum of monthly means for May–October) for the period 1963–76.

TABLE 1. Matrix of correlation coefficients of interstock recruitment trends where R_t = 1963–76 year-classes (logarithmically transformed). $*p < 0.01$; $**p < 0.05$.

	Correlation matrix						
	WB-NDB	BB-TB	CB-SS	SMB-PB	FB	4R	4T
WB-NDB	1.00	0.94**	0.92**	0.83**	0.76**	0.76**	0.52*
BB-TB		1.00	0.96**	0.80**	0.72**	0.79**	0.51*
CB-SS			1.00	0.84**	0.81**	0.87**	0.68**
SMB-PB				1.00	0.84**	0.66*	0.76**
FB					1.00	0.68*	0.84**
4R						1.00	0.61*
4T							1.00

stock-specific recruitment estimates (log-transformed) for the 1963–76 year-classes (Table 1). Generally each stock is better correlated with the adjacent upstream stock than with the adjacent downstream stock with respect to the Labrador Current (Fig. 5). The reason for this pattern is not clear but it may reflect the diminution of the effect of the Labrador Current as it flows southwards along the Newfoundland coast. The relationship of the recruitment patterns among the various herring stocks was also examined through principal component (PC) analysis of the recruitment matrix. The first two PCs explained 81 and 9%, respectively, of the variance of the recruitment data set. Plots of the eigenvectors of PC 1 and PC 2 against each other (Fig. 6) showed that the Gulf of St. Lawrence stocks (4T, 4R) form a separate clumping, which is perhaps not surprising because they are both influenced by the St. Lawrence River discharge, and in addition, tagging studies (Winters and Beckett 1978) have established a minor degree of interchange between these two stocks. The three eastern Newfoundland stocks (White Bay – Notre Dame Bay, Bonavista Bay – Trinity Bay, Conception Bay – Southern Shore) are more closely related in terms of recruitment structure than southern Newfoundland stocks (St. Mary's Bay – Placentia Bay, Fortune Bay).

TABLE 2. Correlation matrix of log-transformed recruitment estimates (1963–76 year-classes) in relation to the various environmental signals (T_1 and S_1-temperature and salinity data for the overwintering/prespawning period; T_2 and S_2-temperature and salinity data for the summer/postspawning period) for spring-spawning herring stocks. Correlation values based on detrended data are shown in parentheses. $*p < 0.01$; $**p < 0.05$.

	Correlation coefficient of $\log_e R_t$ with			
Area	T_1	T_2	S_1	S_2
WB-NDB	0.79** (0.70**)	−0.23	0.26	0.40
BB-TB	0.75** (0.61*)	−0.20	0.29	0.39
CB-SS	0.67** (0.62*)	−0.22	0.39	0.48
SMB-PB	0.56* (0.57*)	−0.38	0.60* (0.63*)	0.49
FB	0.57* (0.69**)	−0.22	0.36	0.33
4R	0.62* (0.56*)	−0.34	0.31	−0.01
4T	0.25	−0.53	0.59* (0.61*)	0.23

Recruitment Variability and Environmental Effects

The synchrony in recruitment of the various spring-spawning herring stocks is consistent with our hypothesis that large-scale oceanographic events are the major underlying determinants of

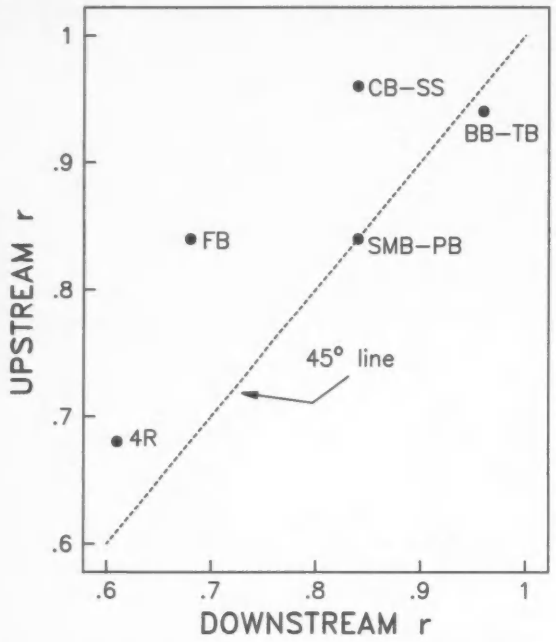

FIG. 5. Relationship between the correlation coefficients of the recruitment (log transformed) of downstream herring stocks with the recruitment of upstream stocks (see text for explanation).

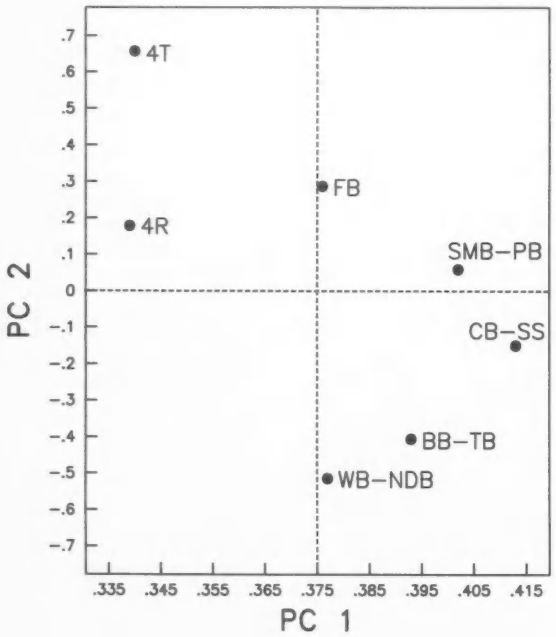

FIG. 6. Principal component (PC) analyses of the recruitment patterns of the various spring-spawning herring stocks in the Northwest Atlantic.

TABLE 3. Estimates of mean-squared errors (MSE) and changes in the multiple correlation coefficients (ΔR^2) as a result of the entry of the various independent variables (T_1, T_2, S_1, S_2) into the multiple stepwise regression model.

	WB-NDB			BB-TB			CB-SS			SMB-PB			FB			4R			4T		
Variable	MSE (df)	ΔR^2	$p\ (F>\hat{F})$	MSE (df)	ΔR^2	$p\ (F>\hat{F})$	MSE (df)	ΔR^2	$p\ (F>\hat{F})$	MSE (df)	ΔR^2	$p\ (F>\hat{F})$	MSE (df)	ΔR^2	$p\ (F>\hat{F})$	MSE (df)	ΔR^2	$p\ (F>\hat{F})$	MSE (df)	ΔR^2	$p\ (F>\hat{F})$
T_1	1.11 (1,12)*	0.64	0.001	1.60 (1,12)*	0.56	0.002	1.69 (1,12)*	0.45	0.009	0.45 (1,12)*	0.48	0.001	1.89 (1,12)*	0.32	0.034	1.33 (1,12)*	0.38	0.018	0.43 (1,11)*	0.14	0.044
T_2	0.64 (1,9)	0.01	>0.500	1.06 (1,9)	0.01	>0.500	0.84 (1,10)	0.01	>0.500	0.42 (1,10)	<0.01	0.445	3.46 (1,9)	<0.01	>0.500	0.89 (1,11)*	0.28	0.023	0.46 (1,10)	0.02	>0.500
S_1	0.63 (1,11)*	0.18	0.008	0.98 (1,11)*	0.20	0.012	0.88 (1,11)*	0.29	0.005	1.67 (1,11)*	0.36	0.023	1.37 (1,11)*	0.23	0.039	0.93 (1,9)	0.04	0.327	0.50 (1,12)*	0.34	0.009
S_2	0.62 (1,10)	0.02	>0.500	1.00 (1,10)	0.02	0.403	0.76 (1,9)	0.06	0.118	0.41 (1,9)	<0.03	0.165	1.48 (1,10)	<0.01	>0.500	0.93 (1,10)	0.02	>0.500	0.49 (1,9)	0.01	>0.500

*Selected variables

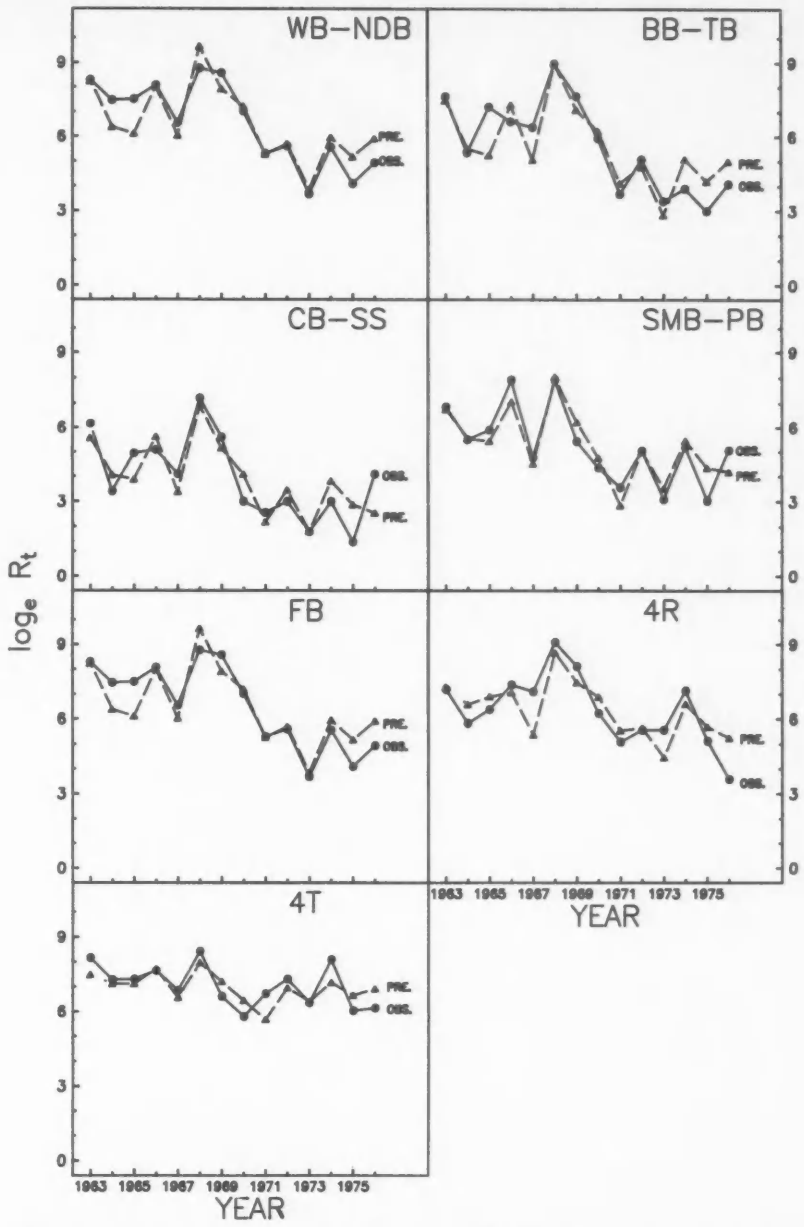

FIG. 7. Comparison of observed recruitment with that predicted from the stock-specific empirical recruitment models for the 1963–76 year-classes.

recruitment variability. In Table 2 we present a matrix of correlation coefficients relating recruitment (log-transformed) of the various stocks to the chosen environmental parameters. There is a significant positive association between overwintering temperatures (i.e. T_1) and recruitment for all stocks except 4T, where overwintering salinity (i.e. S_1) is the significant environmental factor. The results based on detrended data are similar and demonstrate that the relationships are independent of time-series effects. The second important variable is overwintering salinity (S_1) which is significantly related to recruitment for St. Mary's Bay – Placentia Bay in addition to 4T. The correlation coefficients between T_1 and $\log_e R_T$ show a general decrease from north (upstream) to south (downstream) similar to the trend in interstock correlations of recruitment.

Multiple stepwise regression analyses with the criteria of acceptance described previously resulted in the selection of T_1 and S_1 as the independent variables for all stocks (Table 3)

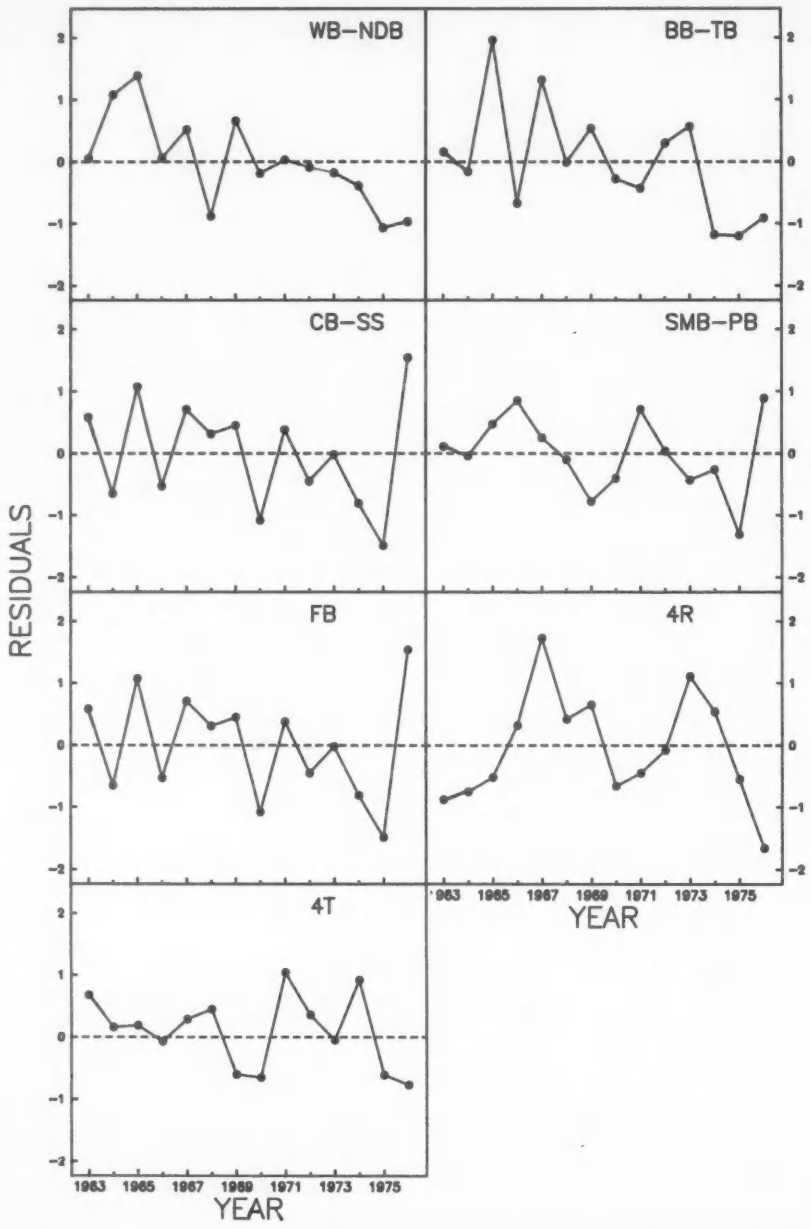

FIG. 8. Lognormal residuals of the empirical recruitment models for the various stocks for the 1963–76 year-classes.

except 4R where T_2 replaced S_1 as the secondary independent variable. The parameter estimates of the empirical recruitment models are given in Table 4.

Development of an Environmentally Dependent Stock–Recruit Model

Comparison of observed recruitment with recruitment levels predicted from the empirical model for each stock (Fig. 7) demonstrated that the empirical models were able to duplicate major changes in recruitment adequately. However, an examination of the pattern of residuals showed that for some stocks (e.g. White Bay – Notre Dame Bay, Bonavista Bay – Trinity Bay, Conception Bay – Southern Shore), the lognormal residuals (Fig. 8) exhibited a nonrandom distribution. This pattern in the residuals may be attributable to other factors which have varied over the time period involved. In nearly all stocks, egg production levels (E) changed dramatically during

TABLE 4. Stock-specific multiple regression models of recruitment ($\log_e$ transformed) versus the two dominant environmental variables.

Stock	Multiple regression model	R^2	SE	Dominant variable
WB-NDB	$\log_e R_T = 0.46T_1 + 4.88S_1 - 152.50$	0.81	0.79	T_1
BB-TB	$\log_e R_t = 0.49T_1 + 5.66S_1 - 178.53$	0.76	0.99	T_1
CB-SS	$\log_e R_t = 0.39T_1 + 6.24S_1 - 197.93$	0.73	0.94	T_1
SMB-PB	$\log_e R_t = 0.34T_1 + 8.08S_1 - 257.06$	0.81	0.78	T_1
FB	$\log_e R_t = 0.33T_1 + 5.28S_1 - 165.921$	0.55	1.17	T_1
4R	$\log_e R_t = 0.32T_1 - 0.18T_2 + 12.48$	0.62	0.94	T_1
4T	$\log_e R_t = 0.10T_1 + 3.80S_1 - 115.3$	0.48	1.66	S_1

TABLE 5. Environmentally dependent recruitment models derived for the various spring-spawning herring stocks in the Northwest Atlantic.

Stock	Empirical recruitment model	R^2	SE of estimate
WB-NDB	$R = Ee^{(-120.80 - 0.0151E + 0.295T_1 + 3.99S_1)}$	0.96	0.52
BB-TB	$R = Ee^{(-174.66 - 0.0224E + 0.263T_1 + 5.66S_1)}$	0.92	0.82
CB-SS	$R = Ee^{(-215.94 - 0.0947E + 0.365T_1 + 6.91S_1)}$	0.88	0.99
SMB-PB	$R = Ee^{(-253.11 - 0.0207E + 0.305T_1 + 8.00S_1)}$	0.85	0.73
FB	$R = Ee^{(-185.84 - 0.0291E + 0.336T_1 + 5.82S_1)}$	0.54	1.24
4R	$R = Ee^{(13.59 - 0.0097E + 0.291T_1 - 0.177T_2)}$	0.69	0.97
4T (1963–76)	$R = Ee^{(-107.94 - 0.0025E + 0.049T_1 + 3.44S_1)}$	0.52	0.67
4T (1969–79)	$R = Ee^{(-128.88 - 0.0115E + 0.186T_1 + 4.11S_1)}$	0.79	0.64

TABLE 6. Comparison of parameter estimates for egg production using the simple Ricker stock–recruit model and the environmentally dependent Ricker model. *$0.01 < p < 0.05$; **$p < 0.01$.

	Simple Ricker			Environmentally dependent Ricker		
Stock	R^2	$-\beta_1$	Egg production ($\times 10^{-11}$) at R_{max} (E_c)	R^2	$-\beta_2$	Egg production ($\times 10^{-11}$) at R_{max} (E_c)
WB-NDB	0.83**	0.021	48	0.96**	0.015	66
BB-TB	0.80**	0.030	33	0.92**	0.022	45
CB-SS	0.64**	0.155	6.5	0.88**	0.095	10.5
SMB-PB	0.17	—	—	0.85**	0.021	48
FB	<0.01	—	—	0.54*	0.029	34
4R	0.38*	0.025	40	0.69**	0.010	103
4T (1963–76)	0.23	—	—	0.52*	0.003	400
4T (1969–79)	0.54*	0.011	95	0.79**	0.012	87

the time period under consideration (Fig. 3). Accordingly, the key environmental variables identified for each stock (Table 4) were included in the environmentally dependent Ricker stock–recruit model. The derived environmentally dependent recruitment models (Table 5) resulted in improved fits for all stocks except Fortune Bay. For comparison purposes, stock–recruit curves were also derived using the simple Ricker model (Ricker 1954) (Table 6). Comparison of estimates of the density-dependent parameter, β, by both models (Fig. 9) indicated that the simple Ricker model has a tendency to overestimate the level of this parameter.

For some stocks, and particularly those along eastern Newfoundland, the environmental Ricker model is characterized by substantial compensatory density dependence such that recruitment at high egg production levels is lowered (Fig. 10). The biological reality of the Ricker curves in Fig. 10 may be seen through comparison of egg production levels at maximum (geometric) recruitment, as estimated by the environmental Ricker model (i.e. the critical spawning stock size (E_c) (Table 6)), with egg levels produced by the observed geometric mean recruitment (E_p), i.e. the level of annual egg production associated with the recruitment level of greatest frequency (E_p). From an evolutionary viewpoint, it is intuitively reasonable that the egg production at which the geometric mean recruitment occurs should closely approximate the egg production at maximum recruitment size. Obviously, if E_p falls significantly below E_c, the stock would become extinct in time due to stochastic events. Comparisons of E_p and E_c values (arrows in Fig. 10) confirm our expectations for all stocks except 4T, where E_p is much lower than E_c. Taken at face value, Fig. 10 suggests that spring-spawning herring in 4T were very vulnerable to recruitment collapse during the late 1960's and 1970's. However, examination of the Ricker model for 4T (Fig. 11) indicates that the 1963–68 data points are

TABLE 7. Coefficients of variation of mean temperature for the period May–July at various localities along eastern and southern Newfoundland and the southern Gulf of St. Lawrence (4T). The Entry Island data (Lauzier and Hull 1969) are from long-term surface temperature data whereas temperature data for the Newfoundland localities are from a depth of 9 m for the period 1974–83.

Locality	Mean temperature (°C)	SD	Coefficient of variation (%)
Comfort Cove, Notre Dame Bay	3.36	1.43	42.6
Stock Cove, Bonavista Bay	4.03	1.17	29.0
Station 27	5.01	1.14	22.8
Arnold's Cove, Placentia Bay	5.78	1.01	17.4
Entry Island (4T)	9.83	1.05	10.7

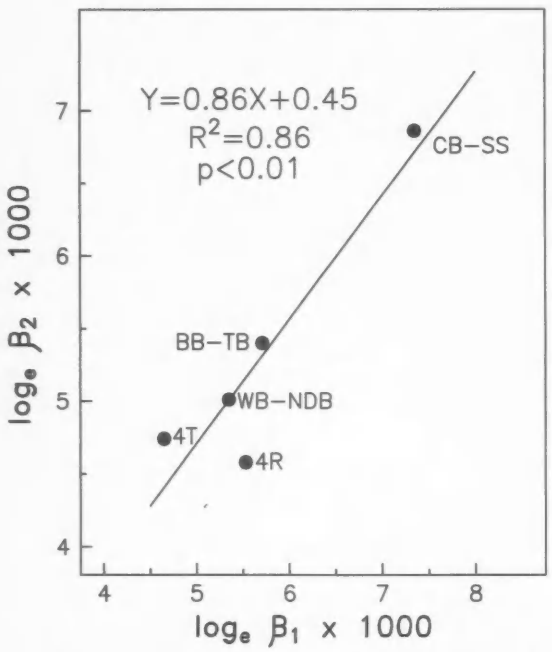

FIG. 9. Relationship between the parameter β of the environmental Ricker model (β_2) and the simple Ricker model (β_1).

clumped at higher levels of egg production. This reflects the impact of the anomalously large 1959 year-class which was nearly an order of magnitude larger than any subsequent year-class (Winters and Hodder 1975). We reanalyzed the Ricker plots for the period after 1968 includng the 1977–79 year-classes. The latter year-classes were relatively insensitive to the input parameters of cohort analysis because they were subjected to high fishing mortalities (Cleary 1984). Highly significant fits were obtained for both the simple Ricker model and the environmentally dependent model (Table 6), and the critical egg production level derived from both models approximates that of E_p Fig. 10; Table 6), which is in accordance with our expectations. We therefore concur with Winters and Hodder (1975) regarding the anomalous events in 4T during the late 1950's and conclude that the stock–recruit curve estimated from the 1970's data set is more representative of 4T herring.

Recruitment Variability and Compensatory Response

The Ricker stock–recruit curves fitted to Northwest Atlantic spring-spawning herring (Fig. 10; Table 6) are characterized by variable but generally high values for the density-dependent parameter, β. This is particularly so for the smaller stocks (e.g. Conception Bay – Southern Shore), which largely depend on a single year-class to sustain their population abundance over time, than for the larger stocks (e.g. 4T, White Bay – Notre Dame Bay), which have a better balance in their year-class composition. We have examined the relationship between the log-transformed estimates of β from the Ricker environmental model (i.e. β_2, Table 6) and mean stock size as measured by the historical mean recruitment. Values of β are inversely related to mean stock size (Fig. 12A). In addition, recruitment variability, as measured by the coefficient of variation, is also inversely related to mean stock size (Fig. 12B), implying that β is a positive function of recruitment variability. Thus, high levels of recruitment variability associated with the smaller herring stocks are compensated for by a high level of recruitment density dependence (as measured by β) and hence a relatively lower critical spawning stock size.

We have shown that environmental variability is the major cause of recruitment variability within stocks. In addition to the effect of stock size on interstock levels in recruitment variability, geographical differences in environmental variability should also affect interstock differences in recruitment variability. A time series of comparable temperature data is available only for the period May–July for selected areas (Table 7) and shows, as expected, that environmental variability (as measured by the coefficient of variation) decreases from north to south along the trajectory of the Labrador Current. These data were included in a multiple regression model to evaluate the relative contribution of mean stock size ($\bar{R}$) and temperature variability ($T_{C.V.}$) to stock-specific levels of recruitment variability ($\bar{R}_{C.V.}$). The addition of $T_{C.V.}$ increased the explained variation in $R_{C.V.}$ from 67 to 86%, and the relationship is described by the following algorithm:

$$R_{C.V.} = 1.78T_{C.V.} - 0.69\,\bar{R} + 178.7 \qquad (R^2 = 0.86).$$

Because temperature variability is relatively greater in the more northern areas of the range of Atlantic herring, we conclude that for different stocks of the same size the density dependence on recruitment will be highest in the more northern populations.

Cushing and Harris (1973) have proposed an alternative index of density dependence of recruitment defined as $-\beta E$ where

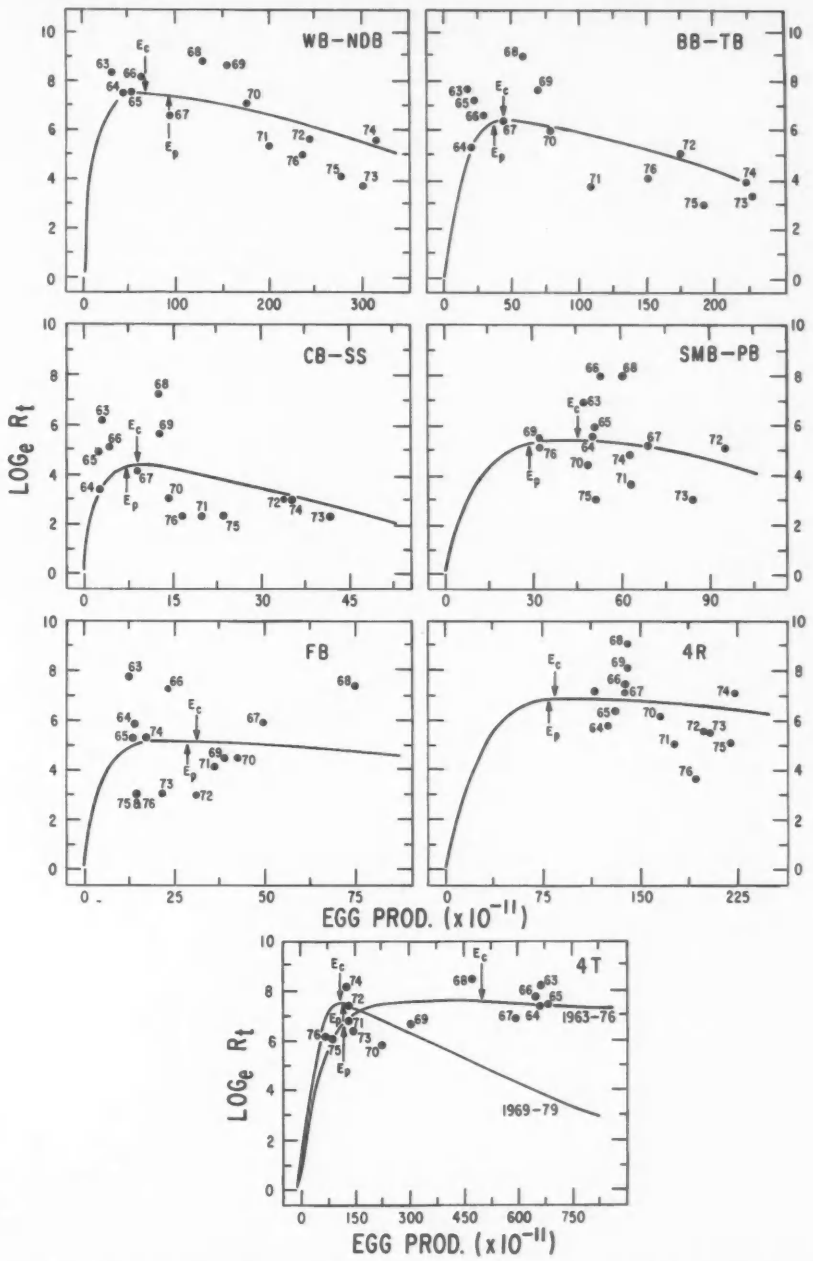

FIG. 10. Ricker stock–recruit curves for the various spring-spawning herring stocks in the Northwest Atlantic (see text for explanation).

E = mean level of egg production, i.e. $-\beta E$ = measure of average recruitment compensation per unit egg production. Table 8 provides estimates of this index for a wide variety of stocks including some stock-specific values of $R_{C.V.}$ Values of βE are lowest for stocks in the central part of their range and which generally have lower levels of recruitment variability. It is not surprising, therefore, that nearly all of the herring stocks which have collapsed because of overfishing (e.g. Arcto-Norwegian, Sakhalin, North Sea) have very low levels of density dependence at the mean level of egg production. Concomitantly, stocks at the northern parts of their range (e.g. Alaska, eastern Newfoundland) have a high degree of recruitment compensation and appear to have a degree of resiliency to exploitation that is perhaps unexpected. This conclusion, how-

TABLE 8. Stock-specific values of Cushing's index of density dependence ($-\beta E$) where β = density-dependent parameters of the simple Ricker Model and E = mean egg production level.

Herring stock	$-\beta E$	Recruitment C.V. (%)	Range location	Source
Alaska	2.10	N/A	Northern	Cushing and Harris (1973)
British Columbia	1.43[a]	34	Central	Stocker et al. (1985)
Sakhalin	0.86[a]	N/A	Central	Cushing and Harris (1973)
North Sea	0.54[a]	N/A	Central	Cushing and Harris (1973)
Arcto-Norwegian	0.25[a]	65	North-central	Cushing and Harris (1973)
Newfoundland				
WB-NDB	3.49	117	Northern	This paper
BB-TB	3.06	186	Northern	This paper
CB-SS	2.64	196	Northern	This paper
4R	4.13	162	Northern	This paper
S. Gulf (4T)	1.38[a]	84	Central	This paper

[a]Stocks that have collapsed or whose fisheries have been closed due to recruitment overfishing.

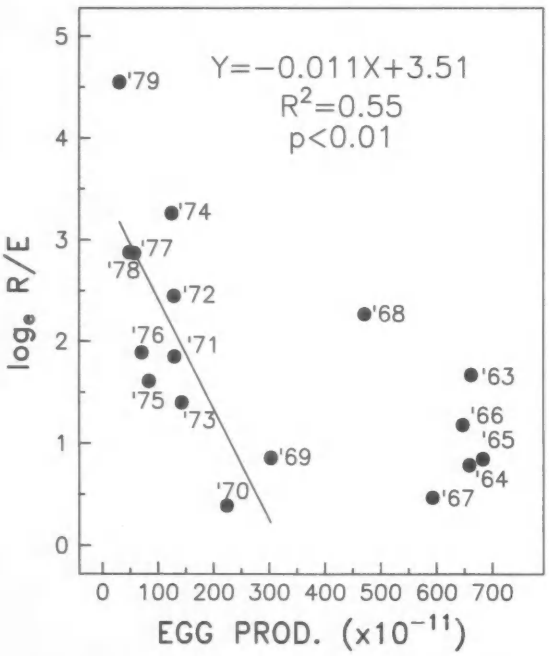

FIG. 11. Regression of $\log_e R/E$ on E for the period 1969–79 for 4T herring.

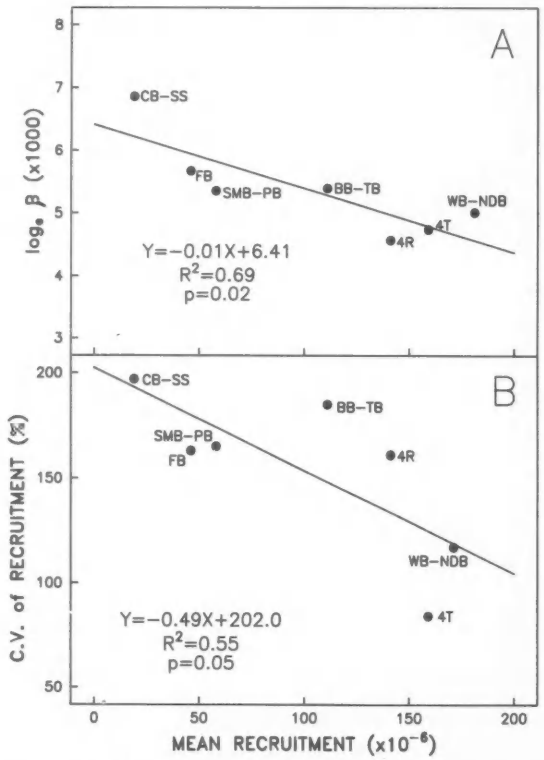

FIG. 12. Relationship between (A) the Ricker parameter β and the mean stock size and (B) the coefficient of variation of mean recruitment and stock size.

ever, is not necessarily valid because recruitment variability will obviously have a large impact on spawning stock dynamics and hence sustainable levels of exploitation. The impact of recruitment variability and stock–recruit dynamics on ability of these herring stocks to sustain particular levels of fishing mortality was assessed by the stochastic simulation model described previously.

Results of Simulation Runs

A listing of data inputs (empirically based) for each stock is given in Table 9 and the resulting yield curves and summary statistics are given in Fig. 13 and Table 10, respectively. In the case of unrestricted fishing (option I), yield curves for all stocks were dome shaped, with the level of fishing mortality producing maximum yields (i.e. F_{max}) varying from a high of $F_{max} = 0.43$ for White Bay – Notre Dame Bay to a low of $F_{max} = 0.22$ for Conception Bay – Southern Shore herring. For option II, yield curves were flat topped for all stocks and asymtotic yields were generally greater and occurred at higher

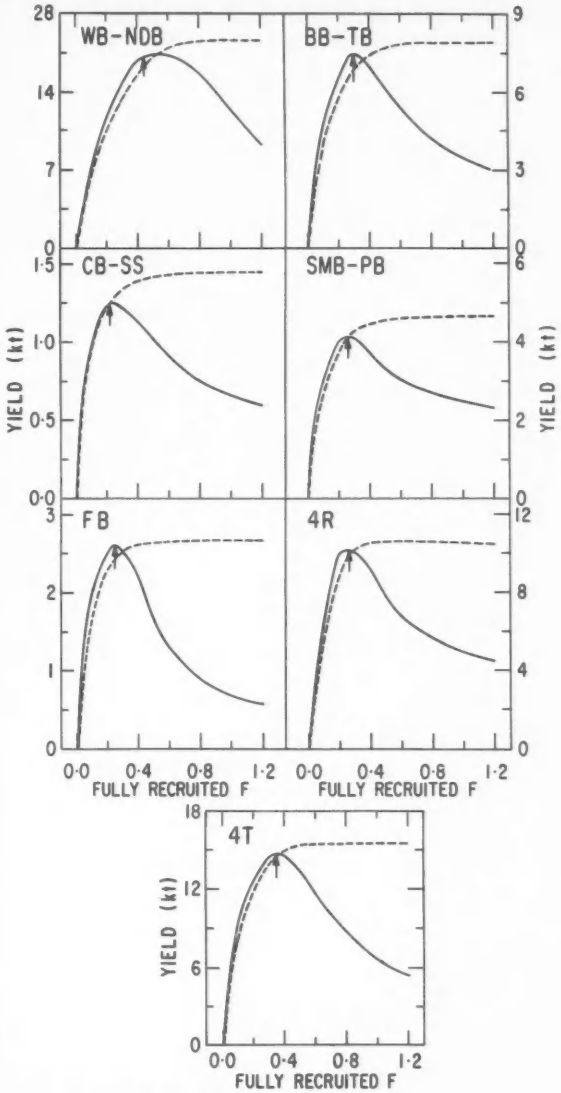

FIG. 13. Simulation model estimates of yield in relation to fully recruited fishing mortality (F) for the various spring-spawning herring stocks in the Northwest Atlantic (solid line represents option I (no spawning stock restraint); broken line represents option II (spawning stock restraint)).

levels of fishing mortality than in the case of unrestricted fishing. Variability in yields (Fig. 14) increased substantially as fishing mortality levels increased but was generally lower for option 2. Under option I, recruitment variability for all stocks was fairly stable at levels of up to $F = 0.30$ (Fig. 14) but increased substantially at higher levels of F. By contrast, recruitment variability was much lower and more stable under option II.

Doubleday (1985) proposed an exploitation rate of 0.20 for herring stocks because this rate would allow at least 70% of maximum yields to be taken at a relatively high level of stock stability (C.V. of biomass of 40% or less). In Table 11 we present simulation model estimates of the percentage of maximum yield and the C.V. of biomass for the various stocks when subjected to a fishing mortality rate of $F = 0.20$. It can be seen that under both fishing strategies, maximum yields of more than 70% would be taken. However, under option II, biomass variability would be substantially less and would approximate the 40% proposed by Doubleday.

We have already demonstrated that recruitment variability in these herring stocks is compensated for by density-dependent changes in the parameter β which has been shown to be an inverse function of mean stock size. Analyses of stock-specific levels of F_{max} (Table 12; Fig. 15) show that the most important predictor of F_{max} is mean stock size, i.e. large stocks can sustain higher levels of fishing mortality than smaller stocks. Recruitment variability is also positively correlated with temperature variability; therefore, for stocks of the same size, the northern stocks should be able to sustain higher levels of fishing mortality than more centrally located stocks. This is consistent with our conclusions from the Cushing and Harris (1973) index of recruitment density dependence.

In summary, we conclude that the ability of a herring stock to withstand fishing mortality is primarily a function of its mean stock size which in turn is the main determinant of its stock–recruit parameters as well as its recruitment variability. Furthermore, we conclude that a management strategy of curtailing fishing as the spawning stock approaches the critical level not only will result in higher long-term yields but these yields will be at much higher levels of fishing effort and with reduced variability in annual recruitment. However, if constant fishing effort is the desired management strategy, a level of $F = 0.20$ is recommended in combination with fishing closures as the spawning stock approaches the critical level.

Discussion and Conclusions

Our analyses of recruitment dynamics among the major spring-spawning herring populations in the Northwest Atlantic have demonstrated recruitment success to be similar among stocks and consistent with the hypothesis that this synchrony is regulated mainly by common oceanographic events, i.e. overwintering temperatures and salinities of the Labrador Current. Removal of the environmental component of recruitment variability resulted in stock–recruit curves whose characteristics included sharply ascending left limbs and high degrees of compensatory density dependence in recruitment. The degree of such density dependence (as measured by the Ricker parameter, β) was directly related to recruitment variability which was itself negatively associated with mean stock size. This finding is intuitively reasonable, since large herring stocks occupy larger areas (Winters and Wheeler 1985) which generally have a greater variety of suitable habitat for larval herring and also larger larval retention areas (Iles and Sinclair 1982). Hence, natural variations in environmental conditions would be considerably dampened. An additional factor, environmental variability, was positively associated with the level of recruitment variability, which is also in accordance with expectations. Simulation studies, using the empirically based recruitment models, showed that large stocks could sustain higher levels of fishing mortality than smaller stocks, due mainly to reduced recruitment variability.

The level of density dependence of recruitment evident from our analysis of the spring-spawning herring stocks of the North-

TABLE 9. Listing of stock-specific data input used in simulation modelling of Northwest Atlantic herring recruitment dynamics. The partial rerecruitment rate for all stocks was 10% (age 2), 40% (age 3), 75% (age 4), and 100% (age 5+). a = chosen randomly; b = long-term average for stock; c = from annual age-specific weights and regression of $\log_e \bar{W}_t$ on fecundity (Hodder 1972).

Parameter	Stock						
	WB-NDB	BB-TB	CB-SS	SMB-PB	FB	4R	4T
Initial age structure	a	a	a	a	a	a	a
Critical spawning stock (no. eggs $\times 10^{-11}$)	66	45	10	48	34	103	87
Age-specific weight	b	b	b	b	b	b	b
Age-specific fecundity	c	c	c	c	c	c	c
Maturation rates	b	b	b	b	b	b	b
Maximum life span (yr)	15	15	15	15	15	15	15
Natural mortality rate (M)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
T_1 ($\bar{x}$, SD) = (6.57, 3.31)							
S_1 ($\bar{x}$, SD) = (32.01, 0.15)							
T_2 ($\bar{x}$, SD) = (45.06, 4.11)							

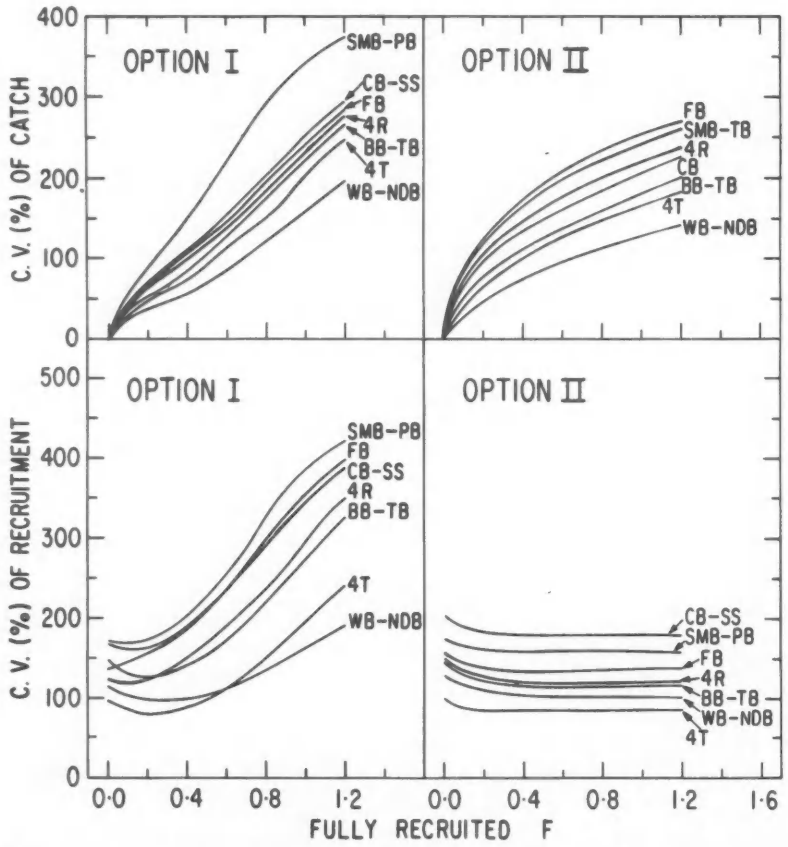

FIG. 14. Simulation model estimates of yield and recruitment variability (as measured by the coefficient of variation) in relation to fully recruited fishing mortality rates for the various spring-spawning herring stocks in the Northwest Atlantic.

west Atlantic is high and is, in fact, higher than comparable estimates for many demersal species which have generally been accepted to be more resilient to exploitation than herring stocks (Cushing and Harris 1973). This is a surprising finding but it is not inconsistent with the high incidence of herring stock collapses due to overfishing. As Burd (1985) has aptly pointed out, an outstanding feature of the biology of herring is their ability to recover from extreme levels of overfishing, and there

TABLE 10. Summary statistics of the simulation model output of each herring stock.

Stock	Option I			Option II
	F_{max}	Yield at F_{max} (kt)	C.V. (%) of yield at F_{max}	Asymptotic yield (kt)
WB-NDB	0.43	15.0	57.5	17.5
BB-TB	0.30	7.3	64.1	7.6
CB-SS	0.22	1.3	67.2	1.7
SMB-PB	0.27	4.1	97.8	4.5
FB	0.23	2.6	56.3	2.7
4R	0.27	9.8	58.8	10.2
4T	0.35	14.6	60.3	14.8

TABLE 11. Simulation model estimates of percentage of maximum yield and coefficient of variation in biomass resulting from a fishing mortality of $F = 0.20$ for the various stocks of spring-spawning herring.

Stock	Option I		Option II	
	C.V. of biomass (%)	$\% \left(\frac{C_t(F=0.20)}{C_t(F_{max})}\right)$	C.V. of biomass (%)	$\% \left(\frac{C_t(F=0.20)}{C_t(F_{max})}\right)$
WB-NDB	33	81	32	77
BB-TB	51	86	41	78
CB-SS	64	94	52	93
SMB-PB	71	98	48	77
FB	46	96	33	73
4R	53	90	38	81
4T	37	86	33	74
Average	51	90	40	79

TABLE 12. Simple correlation analysis of the relationship between stock-specific levels of F_{max} and stock size ($\bar{R}_t$), recruitment variability ($R_{C.V.}$), and the Ricker parameter, β. $^*p < 0.05$.

Independent variable	Correlation coefficient (r) with		
	$\bar{R}_t$	$R_{C.V.}$	β
F_{max}	0.87*	−0.74	−0.58

are many examples in the published literature to support this viewpoint (e.g. Hourston 1980; Jakobsson 1980, 1985). Our analyses suggest that this resilience is primarily due to a general feature of the stock–recruit relationship of herring, i.e. a curve with a rapidly ascending left limb and a relatively low critical spawning stock size. Indeed, as we have already pointed out, this relationship is a necessity for stocks which have a high recruitment variability. Paradoxically, this characteristic also accounts in large part for the high frequency of herring stock collapses, as the sharply rising curve would tend to mask the imminence of recruitment failure, despite drastic reductions in adult stock size levels. Reviews of the exploitation history of herring (Jakobsson 1985; Anthony and Waring 1980; Hourston 1980) indicate that for several years prior to collapse, the stocks in question were subjected to very high levels of fishing mortality (in excess of $F = 1.0$) which drastically reduced adult abundance yet provided no significant indication of recruitment failure.

The stock–recruit relationship of herring has been considered in extensive detail in two recent symposia on herring

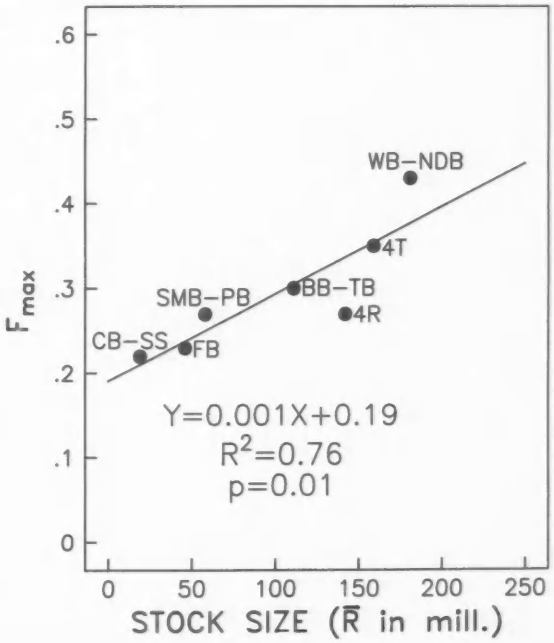

FIG. 15. Relationship between F_{max} and mean stock size of Northwest Atlantic spring-spawning herring.

and pelagic fish (Saville 1980; Brett 1985), but no specific conclusions were reached as to the nature of such relationships or the level of reduction in spawning stock at which fishery closure should be implemented. Indeed, several authors (e.g. Steele 1985) contend that no such relationship exists and that the early life history processes are decoupled from the adult phase. However, as Walters and Ludwig (1981) have pointed out, measurement errors in the estimation of spawning stock and recruitment may mask the existence of any such stock–recruit relationship. For herring stocks, an equally, if not more important consideration is the substantial variability in recruitment due to environmental "noise" (Ulltang 1980) which will also act to obscure the stock–recruit affinity. We have overcome the latter problems through use of an environmental Ricker stock–recruit model (Stocker et al. 1985), and our analyses indicate the stock–recruit relationship of Northwest Atlantic spring-spawning herring to be dome shaped with a high level of density dependence in recruitment. Furthermore, for those stocks that were lightly exploited (and thus approaching virginal levels of egg production) during the period under consideration (e.g. Newfoundland east coast stocks), we determined the critical spawning stock level to be approximately 20% of maximum observed egg production levels. Since the stock–recruit curves of these herring populations were characterized by a rapidly ascending left limb, it would be advisable to implement fishing closure when spawning stock levels fall significantly below 20% of virginal levels. In the absence of data on historical (virginal) population fecundity, our analyses demonstrate that the egg production associated with the geometric mean recruitment will approximate the critical spawning stock size and would therefore be an alternate reference level for closure.

There are several traits associated with herring reproductive ecology which can account for the nature of their stock–recruit relationship and their resiliency to exploitation. By marine fish standards, herring have a fairly large egg (and therefore large larvae), a low fecundity, and a high degree of plasticity to adapt to a wide range of spawning conditions (Blaxter 1985) including a changeover of spawning seasons (Anokhina 1971; Aneer 1985; Winters et al. 1986).

Large eggs and low fecundity are advantageous in areas of poor food/low predator densities whereas small eggs and high fecundity have greater adaptive value in areas richer in food (Hempel and Blaxter 1967). This is reflected in the relative abundance of spring- to fall-spawning herring from north to south in the Northwest Atlantic. Spring spawners have larger eggs and lower fecundities (Hodder 1972) and predominate in the Newfoundland–Labrador area where temperatures are cooler and more variable whereas fall spawners predominate in the more favourable southern areas (Nova Scotia, George's Bank, and the Gulf of Maine). Paulson and Smith (1977) reported a similar cline in egg size/fecundity for Pacific herring.

Another feature that can account for the resiliency of herring stocks to exploitation is density-dependent egg mortality. Both Atlantic and Pacific herring are demersal spawners, and because of a high specificity for certain substrate types, they are limited by the availability of suitable spawning areas (Haegele and Schweigert 1985). It is not unexpected, therefore, that development and survival of egg masses are inversely related to egg density for North American Pacific herring (Taylor 1971; Hourston and Rosenthal 1981; Haegele and Schweigert 1985), Asian herring (Galkina 1961), and Barents Sea herring (Galkina 1971). For example, Taylor (1971) found that an increase in egg deposition could produce an equivalent decrease in hatching success of Pacific herring. Thus, when the spawning stock is large, although there may be a minor extension in the spawning area to less preferred substrates (Taylor 1964), Taylor (1971) concluded that the number of viable larvae produced actually decreased rather than increased. In addition, Hourston et al. (1977) reported that after preferred substrates of British Columbia herring were fully utilized, there appeared to be a change in spawning behaviour wherein eggs were released freely in the water column. Such broadcast spawning would also tend to reduce the relative production of viable larvae. This decrease in viable larvae at heavy spawning intensities explains the dome-shaped reproduction curves for British Columbia herring (Stocker et al. 1985) and Northwest Atlantic spring-spawning herring in our analyses.

An additional mechanism ensures that large changes in spawning stock size are spread evenly throughout the spawning area, thereby optimizing the chances for maximum larval production. Studies (e.g. Winters and Wheeler 1985; Ulltang 1980) have shown that herring have a consistent tendency to maintain school size despite large reductions in abundance. This compensatory behaviour, in the absence of any other mechanism, would imply that egg deposition intensity could remain high even at reduced spawning stock levels, since spawning area would change in direct proportion to changes in population abundance. Recent studies, however, have shown that both Pacific (Hourston 1982) and Atlantic herring (Wheeler and Winters 1984b) have a strong homing tendency to the same spawning grounds, which probably ensures that large changes in population abundance are spread evenly throughout the spawning area. Thus, egg deposition intensity would vary directly with spawning stock size, thereby allowing the stock to compensate for natural fluctuations in abundance through density-dependent changes in egg/recruit survival. This is similar to Atlantic and Pacific salmon which also have a marked homing tendency and, like herring, are limited in the availability of suitable spawning substrate (Ricker 1954; Elson 1975). Recent empirical studies (Jessop 1984) support previous analyses (Elson 1975; Symons 1979; Buck and Hay 1984) that there is substantial density-dependent mortality between the egg to 0+ parr stage producing a dome-shaped stock–recruit curve for salmon (Ricker 1954).

Finally, a very significant finding of our analyses is that herring stocks located at the northern extreme of their range are probably more resilient to exploitation than southern stocks of the same size. This resilience is primarily due to compensatory reductions in the relative size of the spawning stock needed to produce maximum recruitment, and hence, such northern stocks have a much higher degree of redundancy in egg production than herring stocks located in the central part of their range. This finding has significant implications considering that the larger herring stocks are generally located in the central part of the range (e.g. 4T herring) and are really stock "complexes" composed of many individual "stocklets". The variance in the recruitment of the stock "complex" will undoubtedly be lower than that of the individual "stocklet." Mixed stock fisheries, which unequally exploit the individual components, may result in the sequential decimation of these stocklets at levels of fishing mortality assumed to be sustainable from the relationship between F_{max} and mean stock size (see Fig. 15). This possibility adds further support to the proposal of Doubleday (1985) that herring stocks be fished at levels of fishing mortality of $F = 0.20$.

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NOTES

Age Validation for the Mountain Brook Lamprey, *Ichthyomyzon greeleyi*

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Medland, T. E., and F. W. H. Beamish. 1987. Age validation for the mountain brook lamprey, *Ichthyomyzon greeleyi*. Can. J. Fish. Aquat. Sci. 44: 901–904.

Larval mountain brook lamprey, *Ichthyomyzon greeleyi*, were collected from Bent Creek in western North Carolina to provide information on the age structure of the population and to validate statolith banding patterns as an aging technique. Larvae were injected with oxytetracycline in winter and held in the laboratory at seasonally adjusted temperatures for 1 yr. Statoliths from these larvae were examined over the course of the study for the presence and persistence of oxytetracycline, and banding patterns which appeared after marking. Oxytetracycline persisted in all statoliths over the course of the study, and one complete annulus was formed on each statolith during the winter period when growth was slow. The larval period of *I. greeleyi* commences after hatching in late June and persists for 4.2–5.2 yr, with the oldest individuals being predominantly female.

Les auteurs ont recueilli des larves de lamproie de montagnes (*Ichtyomyzon greeleyi*) dans le ruisseau Bent dans l'ouest de la Caroline du Nord afin de fournir des données sur la structure démographique des âges et de valider les anneaux déposés dans les statolites comme méthode de détermination de l'âge. Ils ont injecté aux larves de l'oxytétracycline en hiver et les ont gardées en laboratoire pendant 1 an à des températures ajustées selon la saison. Tout au cours de l'étude, ils ont examiné les statolites afin de déterminer la présence et la persistance de l'oxytétracycline et des anneaux apparus après l'injection. L'oxytétracycline est restée dans tous les statolites pendant l'étude et un anneau complet a été formé sur chacun d'eux pendant l'hiver quand la croissance était lente. Chez *I. greeleyi*, la période larvaire commence après l'éclosion à la fin juin et dure de 4,2 à 5,2 a; les plus vieux individus sont surtout des femelles.

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Discrimination of age in larval lamprey has relied on an analysis of length frequency distributions from large numbers of animals. This technique appears satisfactory for the young age classes, but slower and more variable growth rates in older fish result in greater overlapping of length distributions corresponding to year classes (Potter 1980).

Hard tissue including scales, fin rays, and otoliths are typically used to age teleost fishes. Recognition of annular patterns requires an understanding of how these patterns relate to annual growth cycles in teleosts. In temperate latitudes, extremes in growth imposed by sharp seasonal changes in temperature are manifested in hard tissue by the formation of annuli (Beamish and McFarlane 1983). Structures analogous to the teleost otoliths were described for lamprey as early as 1912 (Studnicka 1912). An internal banding pattern within the statolith was recorded by Carlstrom (1963), and recently, Volk (1986) described a positive relationship between statolith band number and length of larval sea lamprey, *Petromyzon marinus*.

Oxytetracycline injected into the body cavity of fishes is deposited in calcium-containing tissues (Jensen and Cumming 1967; Campana and Neilson 1982; Campana 1983) and is detectable in bone tissue by fluorescence microscopy (Milch et al. 1957). The persistence of oxytetracycline in statoliths has not been investigated in lamprey, although fluorescence bands have been retained in the hard tissues of teleosts for several years (Beamish and McFarlane 1983).

The objective of the current study was to evaluate statolith banding patterns as an aging technique and to provide information on the age structure of the mountain brook lamprey, *Ichthyomyzon greeleyi*, in a small stream in western North Carolina, USA.

Materials and Methods

Larval mountain brook lamprey were electrofished from Bent Creek, a small stream tributary to the French Broad River in western North Carolina (Beamish and Austin 1985), in the summer of 1984. After capture, lampreys (n = 130) were anesthetized in methane tricaine sulfonate (Sandoz), measured to the nearest millimetre, and frozen for later aging. Twenty-two live lamprey ranging in length from 54 to 161 mm were returned to the laboratory. In January they were anesthetized, measured to the nearest millimetre, weighed to the nearest

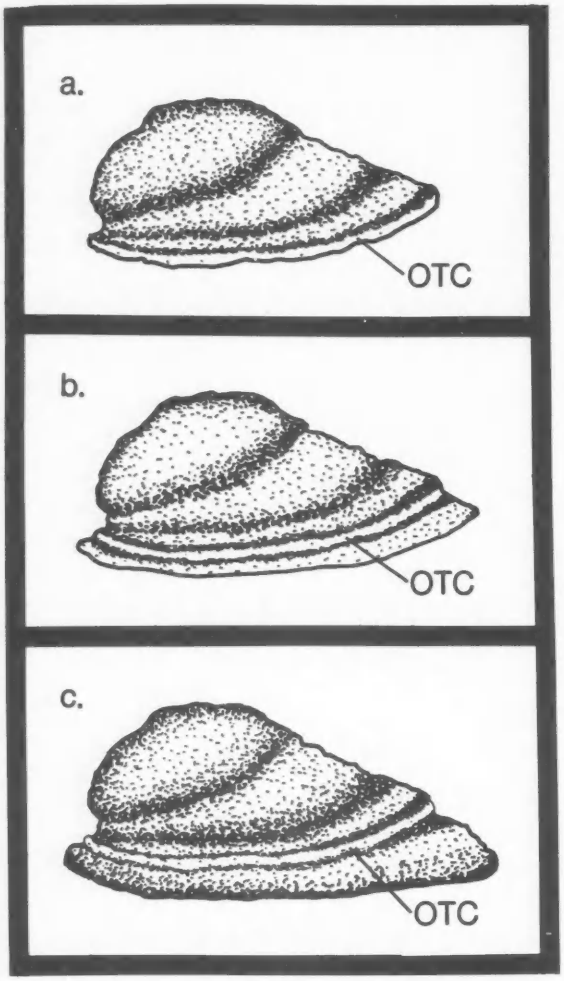

FIG. 1. Lateral view of statoliths from *Ichthyomyzon greeleyi* marked with oxytetracycline (OTC). (a) Six weeks after injection of OTC, a fluoresent band of OTC has been incorporated within the third annulus being formed on the statolith margin; (b) 9 mo after injection, an opaque zone of growth was present on the margin of the statolith beyond the area marked with OTC; (c) 12 mo after injection, a fourth annulus formed on the statolith, with the OTC mark in the third annulus still clearly visible.

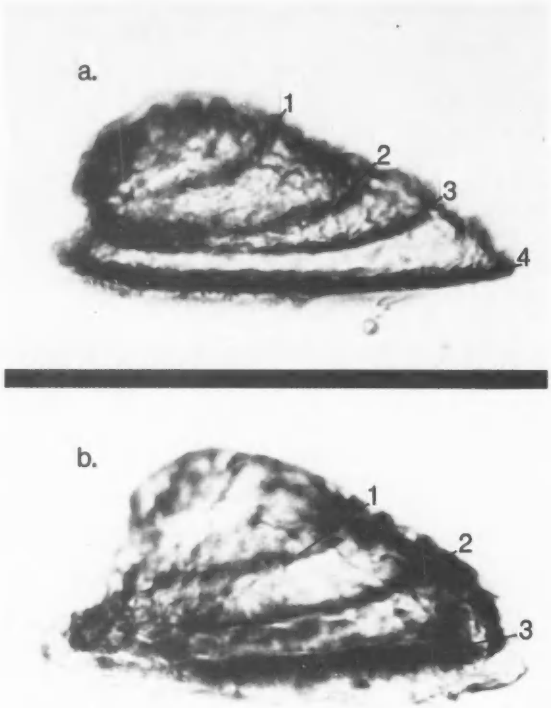

FIG. 2. Lateral view of statoliths from *Ichthyomyzon greeleyi*. (a) Statolith sampled in January showing four annuli; (b) statolith sampled in July showing three annuli followed by an opaque zone of growth.

milligram, and given intraperitoneal injections of oxytetracycline at 35 mg oxytetracycline/kg live body weight. Lamprey were supplied with a suitable substrate for burrowing, a continuous flow of aerated, nonchlorinated groundwater, and fed brewers yeast (100 g dry yeast/22 lamprey) once weekly. Water flow to the tank was stopped for 24 h following feeding. Water temperature was cycled to mimic seasonal temperatures in the lamprey's natal stream (Beamish and Austin 1985). Fish were exposed to a daily photoperiod of 15 h with 0.5 h of gradual brightening or dimming at either end. Surface light intensity at the uncovered portion of the holding tank was approximately 60 lx. This was not varied seasonally.

Lampreys were removed from the holding tank 6 wk (n = 3), 9 mo (n = 3), and 12 mo (n = 16) after injection with oxytetracycline, anesthetized, measured (± 1 mm), and their statoliths removed. All statoliths collected over the course of the study were examined under a dissecting microscope for the presence and persistence of oxytetracycline, as well as banding patterns. Statoliths from frozen animals (n = 130) were also removed, stored in immersion oil for 30 d, and the number of bands counted with a dissecting microscope, as described by Volk (1986). The precision of this technique was calculated using the following index of average error:

$$\frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right]\times 100$$

where N is the number of lamprey aged, R is the number of times each is aged, X_{ij} is the ith age determination of the jth lamprey, and X_j is the average age calculated for the jth lamprey (Chilton and Beamish 1982).

Sex of each larva above 45 mm was determined by visual internal inspection of the gonad under a dissection microscope. Histological examinations were periodically conducted on gonads of animals to confirm identifications made by visual inspection.

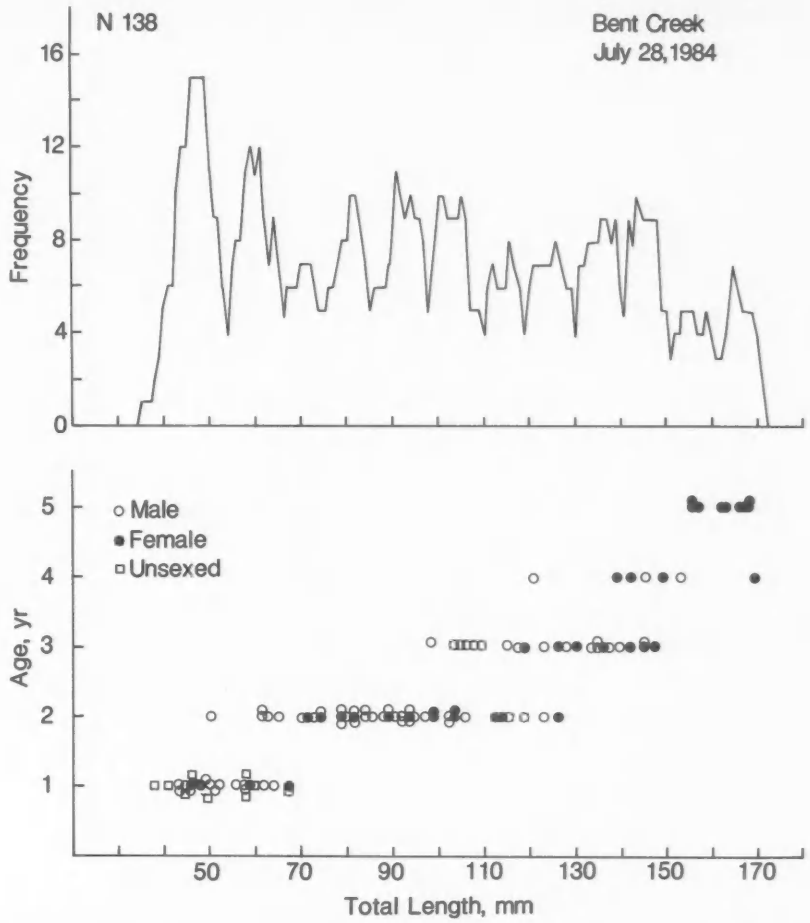

FIG. 3. Length frequency distribution (upper panel) and age (lower panel) of a sample *Ichthyomyzon greeleyi* larvae from Bent Creek, Buncombe County, North Carolina. The length frequency distribution in the upper panel illustrates the difficulty sometimes encountered in identifying age groups. Age of individual larvae determined from statolith banding patterns is described in relation to total length and sex in the lower panel.

Results and Discussion

In ectothermic animals, growth is a manifestation of biotic and abiotic conditions (Beamish and Potter 1975). In temperate climates, growth is particularly responsive to sharp seasonal changes in ambient temperature. Other environmental factors have been demonstrated to affect growth rates in teleost fishes (Beamish and Potter 1975). Fluctuations in biotic and abiotic factors other than temperature may also influence statolith growth but presumably not the number of annuli.

Larvae injected with oxytetracycline in January burrowed in the substrate of the holding tank within 24 h after injection. Statoliths examined in March, 6 wk after injection of oxytetracycline, fluoresced along their outer margins (Fig. 1a) whereas no fluorescence was observed in lampreys not injected. Oxytetracycline persisted in the statoliths throughout the subsequent 11 mo. Nine months after injection, a zone of growth was present on the margin of the statolith beyond the area marked with oxytetracycline (Fig. 1b). Twelve months after injection, a complete annulus had formed on the statolith immediately beyond the area marked with oxytetracycline (Fig. 1c).

When a statolith is oriented properly to transmit incident light, bands appear as layers within the statolith (Fig. 2). Growth of larval *I. greeleyi* is most rapid during the spring and summer (Potter and Bailey 1972; Beamish and Austin 1985). This period of rapid growth is manifested in the statolith as an opaque zone laid down after the narrow darker band produced in the winter. This narrow dark band represents one annulus. Approximate age is equivalent to the number of annuli. Actual age can be calculated from information on the number of annuli and capture date and assuming larvae hatch between early and mid-June (Beamish and Austin 1985).

Mountain brook lamprey larvae captured on July 28, 1984, from Bent Creek ranged in length from 37 to 173 mm (Fig. 3). Among female larvae, five age groups were observed, while only four age groups of males were found. Spawning occurs

between mid-May and early June (Raney 1939; F. W. H. Beamish, unpubl. information). Larvae hatch between early and mid-June, and metamorphosis commences in August (Beamish and Austin 1985). Hence, the current study suggests that the larval interval of mountain brook lamprey ranges between 4.2 and 5.2 yr. The modal lengths of age groups I–IV determined by Beamish and Austin (1985) using length frequency distributions correspond closely with the findings of the current study. However, Beamish and Austin (1985) concluded that the larval period of *I. greeleyi* is 1 yr longer than this study has suggested. This discrepancy occurs in the last larval age group. Beamish and Austin (1985) suggested that a small group of larger, predominantly female larvae belong to a sixth age group, while in the current study, females of equivalent size were found to be 1 yr younger.

The index of average error of aging for this population of *I. greeleyi* was 2.4 (Beamish and McFarlane 1983). This indicates that the repeated aging of one individual in this population would result in the assignment of the same age to that individual 97.6% of the time. The index of average error did not increase significantly ($P = 0.05$) with increasing age of lamprey. Volk (1986) found that this aging technique was accurate for lamprey to 4 yr of age but the application for older animals was not defined by his study. In the current study, this aging technique was validated for lamprey to 5+ yr of age with a highly significant level of precision.

Many studies have demonstrated that body size and the accumulation of energy reserves, particularly lipid, are important to the commencement of metamorphosis, the nontrophic interval between the larval and juvenile period (Farmer and Beamish 1973; Moore and Potter 1976; Beamish and Legrow 1983). While size appears to be important to the commencement of metamorphosis, some large larvae remain as larvae for an additional year, presumably because they have not accumulated sufficient energy reserves (Beamish and Legrow 1983). Both the current study and that by Beamish and Austin (1985) illustrate the fact that the larger and older *I. greeleyi* larvae found in Bent Creek are predominantly female. This would suggest that on average a greater number of years is required for females to accumulate the threshold energy levels required to undergo metamorphosis.

Quantification of age from statoliths removes uncertainties inherent in estimating the age of lamprey populations from length frequency distributions (Fig. 3). This is particularly important in distinguishing age of long-lived species where overlapping of length distributions corresponding to year classes is a common occurrence. The ability to determine the length of the larval period and the variation in larval life within a population may have important applications in the control of some parasitic species.

Acknowledgements

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Selection of Dosages of Oxytetracycline for Age Validation Studies

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McFarlane, G. A., and R. J. Beamish. 1987. Selection of dosages of oxytetracycline for age validation studies. Can. J. Fish. Aquat. Sci. 44: 905–909.

Oxytetracycline (OTC), injected to mark the otoliths of sablefish (*Anoplopoma fimbria*), causes mortality directly in proportion to the dosage administered. The selection of an appropriate dosage requires minimizing mortality while maximizing the number of fish that have a usable OTC mark. Laboratory studies could not be used to determine appropriate dosages. Dosages that substantially increased mortality in the ocean did not cause any mortality in the laboratory. For sablefish, we recommend a dosage of 25–35 mg OTC/kg fish.

L'oxytétracycline (OTC), injectée pour le marquage des otolithes de morue charbonnière (*Anoplopoma fimbria*), entraîne une mortalité directement proportionnelle à la dose. La dose appropriée permettrait de minimiser la mortalité tout en maximisant le nombre de poissons porteurs d'une marque à l'OTC. Des études en laboratoire n'ont pu servir à déterminer les doses appropriées. Les doses qui entraînent un accroissement net de la mortalité en mer n'ont pas été la cause de mortalité en laboratoire. Dans le cas de la morue charbonnière, les auteurs recommandent une dose de 25 à 35 mg d'OTC par kilogramme de poisson.

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The possibility that fish may be substantially older than previously thought (Beamish and McFarlane 1983a, 1986) has resulted in renewed interest in age validation. When the otolith is used to age older, slower-growing fish, validation can only be achieved by marking the otolith. Oxytetracycline (OTC) is commonly used for this purpose because it binds with proteins in the blood and is incorporated in newly forming and mineralizing bone and cartilage (Frost et al. 1961). It is known that high dosages of OTC will cause mortality (Weber and Ridgeway 1962; Kobayashi et al. 1964). It is therefore necessary to select a dosage that provides a suitable mark on the otolith while minimizing mortality.

We injected sablefish (*Anoplopoma fimbria*) with dosages of OTC that were recommended in other studies and found that these dosages did not cause mortality in our laboratory studies (Lanzing and Hynd 1966; Weber and Ridgeway 1967; Wild and Foreman 1980; Beamish et al. 1983). However, in field tests the recapture percentage of the injected fish was much lower than for uninjected fish, indicating that additional mortality was occurring in the ocean (Beamish et al. 1983).

As a result of this unexpected mortality, we conducted a series of experiments in the ocean to determine optimal dosages of OTC for marking otoliths of sablefish. A sufficient number of injected and uninjected fish have now been recaptured to determine an optimal dosage for marking otoliths for age validation studies for this species.

Methods

Initially we followed the procedure of Weber and Ridgway (1962, 1967) and Kobayashi et al. (1964) which indicated that intraperitoneal (IP) injections of OTC at a rate of 20–100 mg/kg fish would result in visible marks. The intensity of the mark on otoliths was a function of the dose administered. Kobayashi et al. (1964) recommended the use of 50 mg OTC/kg fish for young goldfish and Weber and Ridgway (1962) recommended 100 mg OTC/kg fish for young salmon. Both reports noted that mortality increased at dosages exceeding 100 mg OTC/kg fish. Mortalities increased from 0–20% at 100 mg OTC/kg fish to 50–75% at 400 mg OTC/kg fish for goldfish and 50% at 400 mg OTC/kg fish for salmon.

OTC was purchased under the brand name Liquamycin. Liquamycin is oxytetracycline hydrochloride dissolved in a stabilizing agent and supplied in 250-mL bottles at a concentration of 100 mg/mL (Jones 1969). A shallow V-shaped tagging board was calibrated using a weight–length relationship so that the amount of injection could be determined directly from the length measurement. All fish receiving an injection were tagged using the procedures described in Beamish and McFarlane (1983b).

Laboratory studies — In 1977, prior to initiating the tagging program, five adult sablefish were caught by bottom trawls, transported to flowing saltwater-holding tanks in the laboratory, measured, tagged, and injected with OTC concentrations of 50 and 100 mg/kg fish. Fish were maintained at ambient water temperature and fed frozen herring. One fish which received 50 mg OTC/kg fish was sacrificed in the first year and the other four fish survived at least 1 more yr. In April 1978, the experiment was repeated and six adult sablefish were injected with a dosage of 100 mg/kg.

In May and June 1979, 104 adult sablefish were captured at normal fishing depths on the west coast of Vancouver Island and transported to a 92 000-L tank at the Pacific Biological

TABLE 1. Numbers of sablefish surviving, by OTC dosage and time, which were held in the laboratory.

	100 mg/kg	25 mg/kg	Control
Adult sablefish, June 1979 – April 1980			
Sample size	34	34	36
No. surviving after 1 wk	16	16	20
No. surviving after 2 wk	12	12	17
No. surviving after 22 wk	11	12	12
No. surviving after 40 wk	11	10	12
Juvenile sablefish, December 1979 – May 1982			
Sample size	20	20	40
No. surviving after 54 wk	19	19	34
No. surviving after 110 wk	13	17	22

TABLE 2. Recovery rates (%), by OTC dosage of sablefish tagged and released at sea during 1981 (recoveries through December 1985).

Dosage	No. released	No. recovered	% recovered	% (arcsine transformed)
No injection	2118	268	12.6	
1% saline	1484	244	16.4	23.89
25 mg/kg	3266	355	11.0	19.37
50 mg/kg	1010	74	7.3	15.68
75 mg/kg	1071	36	3.4	10.63
100 mg/kg	1481	54	3.6	10.94

Station. One third of the fish received an injection of 100 mg OTC/kg fish, one third were given the same volume of OTC diluted with saline to produce one quarter of the concentration (25 mg/kg fish), and one third were not injected. On December 13, 1979, and on April 16, 1980, all adults in the tank were counted and measured.

Eighty juvenile sablefish were captured in Hecate Strait and Queen Charlotte Sound during September, October, and November 1979 using bottom trawls. They were transported to the same 92 000-L tank at the Pacific Biological Station. On December 13, 1979, one quarter of these fish were injected with 100 mg OTC/kg fish, one quarter were injected with the same volume of solution diluted with saline to produce 25 mg OTC/kg fish, and one half received no injections. All juvenile sablefish were measured every 3 mo until May 1982.

Seawater of ambient temperature was piped into the 92 000-L tank. No attempt was made to regulate the light cycle, but one half of the tank was covered with black plastic to provide some dark areas and lights in the room were turned out at 18:00 each day. No treatment of any kind was provided for disease or injury. All fish were fed frozen herring daily.

Ocean studies — Fish tagged and released off Vancouver Island in 1977 and in 1978 off Vancouver Island and the Queen Charlotte Islands were given an IP injection of OTC amounting to 100 mg/kg fish.

To examine the effect of various dosages on sablefish released directly into the ocean, sablefish captured in March and June 1981 off the west coast of the Queen Charlotte Islands were injected and released. Approximately one quarter were injected with 100 mg OTC/kg fish, one quarter were given the same volume diluted with 1% saline to produce one quarter of the concentration (25 mg OTC/kg fish), one quarter were injected with the same volume of 1% saline solution, and one quarter were not injected.

Sablefish were also collected in November 1981 off the west coast of the Queen Charlotte Islands. Volumes were standardized to the March and June dosage of 100 mg OTC/kg fish. One quarter of the sablefish were given an IP injection of 25 mg OTC/kg fish, one quarter received (IP) 50 mg OTC/kg fish, one quarter received (IP) 75 mg OTC/kg fish, one eighth received no injection, and one eighth were given an intramuscular (IM) injection of 25 mg OTC/kg fish.

Oxytetracycline marks in bone were viewed using ultraviolet light as described by Kobayashi et al. (1964). The presence or absence of a usable mark was noted for all otoliths that were recovered from tagged and injected sablefish. If a mark was sufficiently faint to cause doubt that it was an OTC mark, it was considered to be absent.

Results

In 1977 and 1978, 15 183 fish were tagged, injected with 100 mg OTC/kg fish, and released off the west coast of Canada. As of December 31, 1985, 959 (6.3%) of these fish were recaptured. At the same time, 5279 fish were tagged in the same areas but were not injected. As of December 31, 1985, 1347 (25%) of these fish were recovered.

Laboratory studies — There were no mortalities, as of April 1979, in the two initial laboratory studies. In the laboratory experiments that used adult sablefish to examine the effect of 100 and 25 mg/kg dosages, most mortalities occurred in the first week (Table 1). There was no difference (x^2, $p \geq 0.05$) in the number of mortalities among the dosages after 1 wk indicating that mortalities probably resulted from the stress of capture and transport to the laboratory. When the experiment was terminated after 10 mo, there was no significant difference in the number of fish remaining in any of the groups (χ^2, $p \geq 0.05$).

The laboratory experiment to examine the effect of OTC dosages on mortality and growth of juveniles (Beamish et al. 1983) was completed in 1982 (Table 1). There was no immediate mortality associated with either treatment, and while some mortalities occurred later, there was no significant difference among treatments (x^2, $p \geq 0.05$) after 1 yr (54 wk). After approximately 2 yr (110 wk), there was no significant difference between the highest dosage (100 mg/kg) and the control fish. The fish receiving 25 mg OTC/kg fish had significantly (x^2, $p < 0.05$) less mortality than the control.

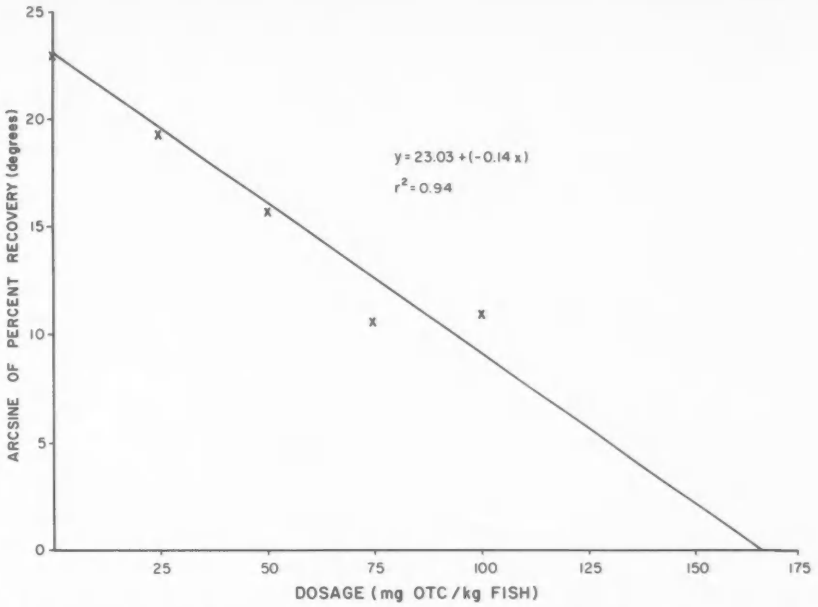

FIG. 1. Relationship between dosage rate of OTC and mortality for tagged and injected sablefish.

TABLE 3. Incidence (%) of OTC marks on otoliths of adult and juvenile sablefish, by OTC dosage and years at liberty, which were tagged, injected, and released at sea in 1981.

Years at liberty	Dosage rate (mg OTC/kg fish)											
	25			50			75			100		
	No. examined	No. with marks	% marked	No. examined	No. with marks	% marked	No. examined	No. with marks	% marked	No. examined	No. with marks	% marked
1	63	37	59	22	18	82	10	9	90	8	6	75
2	87	58	67	24	21	88	17	17	100	21	20	95
3	60	50	83	7	5	71	5	5	100	9	8	89
4	36	27	75	3	2	67	3	2	67	3	3	100
5	8	6	75	—	—	—	—	—	—	1	1	100
Total	254	178	70	56	46	82	35	33	94	42	38	90

Although there was no explanation for the difference between control and 25 mg OTC/kg fish after 2 yr, it is probable that the difference was unrelated to dosage because no significant difference occurred among treatments after 1 yr.

Ocean studies — There was no difference (x^2, $p \geq 0.05$) in the recovery percentages of fish that received IM (9.6%) and IP (11.2%) injections (25 mg OTC/kg fish); therefore, these recoveries were combined (Table 2). The control and 1% saline injections were significantly different (x^2, $p < 0.05$); therefore, their two dosages could not be combined. Because the recovery percentage for fish receiving 1% saline injections was higher, and the treatment procedure for these fish was identical to the procedure for fish receiving OTC injections, this percentage was used in the analysis.

Since percentages are not distributed normally, the values were arcsine transformed (Snedecor and Cochran 1967). A straight line best described the relationship between dosage and mortality (Fig. 1), indicating that mortality is directly related to dosage. An extrapolation of the line in Fig. 1 indicates that a dosage of ~165 mg/kg would appear to cause 100% mortality. There was no indication that a very small dosage would eliminate mortality, that is, no "safe" dosage was found.

The OTC mark occurred less frequently in otoliths from fish that received 25 mg OTC/kg fish than those receiving the higher dosages (Table 3). There was no significant difference (x^2, $p \geq 0.05$) in the percentage of marks (average 92%) resulting from the two highest dosages.

The optimal dosage is that dosage which provides the maximum return of marked otoliths. This dosage was estimated (Fig. 2) and found to be 25–35 mg OTC/kg fish. It is apparent that while smaller dosages resulted in less mortality, they yielded substantially fewer otoliths with an OTC mark. It was only extreme dosages that greatly reduced the probability of recovering otoliths that were usable. These dosages either resulted in no marks or caused high mortality.

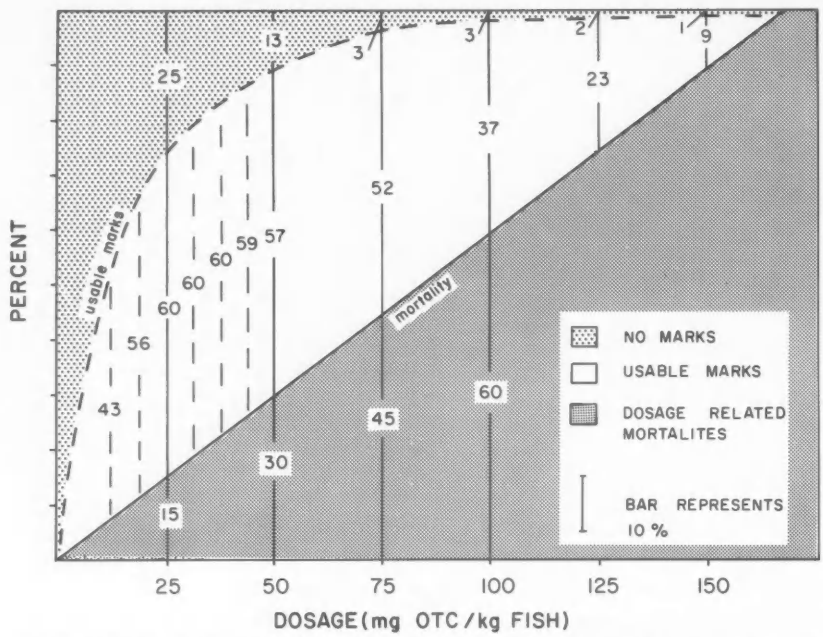

FIG. 2. Nomogram of optimal dosage. Percentage mortality from different dosages was determined from Fig. 1. The percentage of usable marks for each dosage was determined using the percentages in Table 3. For example, a dosage of 25 mg OTC/kg fish causes 15% mortality (Fig. 1), and 70% of the survivers or 60% have usable marks (Table 3). The remainder do not have a mark.

Discussion

The relationship between dosage and survival of injected fish released directly into the ocean was positive and strongly linear. By extrapolation, a dosage of 165 mg OTC/kg fish would likely result in 100% mortality. Because even the smallest dosages cause some mortality, there was no absolutely safe dosage. The relationship between dosage and the percentage of fish that had usable marks, based on recaptures, was not linear. Dosages between 20 and 80 mg OTC/kg fish would produce estimated recoveries up to 75% of those likely to occur with the optimal dosage of 25–35 mg OTC/kg fish. The range of dosages that will produce a usable mark is quite wide and may indicate that dosages close to the optimal for sablefish would be suitable for other species.

The mortality of injected fish observed in the ocean did not occur in any of the laboratory studies. The cause of reduced survival in the ocean, compared with the laboratory, is unknown. However, the fish released in the ocean were tagged and injected immediately after capture, but laboratory fish were allowed to acclimate to the holding tank before injection. Therefore, the two groups may have been stressed differently. In addition, the ambient temperature for fish injected in the laboratory experiments may have been higher than for fish returned directly to the ocean. The reduced metabolic rate of fish returned to the ocean would increase the residence time of OTC and possibly affect survival. Whatever the causes, our study indicates that the laboratory studies did not simulate the conditions affecting injected fish in the ocean and therefore were not controls for the ocean releases. The dosages considered acceptable by other investigators, for other species (Weber and Ridgway 1962, 1967; Kobayashi et al. 1964), were determined in the laboratory. Because our study showed that higher dosages that were safe for laboratory studies caused mortalities in the ocean, it is possible that the higher dosages reported safe for these other species may also cause increased mortality in field experiments. Until the causes of mortality are better understood, the optimum dosage should only be determined from field experiments.

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Analysis of 2-Methylisoborneol in Water, Mud, and Channel Catfish (*Ictalurus punctatus*) from Commercial Culture Ponds in Mississippi

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We describe a new method for the isolation of 2-methylisoborneol (MIB) from channel catfish (*Ictalurus punctatus*) that have been declared "off-flavor" organoleptically. The method involves microwave cooking fish under a nitrogen stream and trapping the condensate at −80°C. The fish condensate was extracted with hexane which was reduced to 100 μL and analyzed by gas chromatography. Concentrations as low as 5 ng MIB·g fish^{-1} were routine detected. A bioconcentration value for MIB in off-flavored fish flesh was 28.1 ± 14.0 (±SD) concentration water/concentration fish. Analysis of off-flavored fish collected at the processing plant consistently showed elevated concentrations of MIB. Geosmin, another compound known to cause flavor and odor problems in fish flesh, was not detected.

Les auteurs décrivent une nouvelle méthode pour l'isolation du 2-méthylisobornéol (MIB) de barbues de rivière (*Ictalurus punctatus*) dont le mauvais goût a été révélé par des tests organoleptiques. La méthode comprend la cuisson du poisson sous un jet d'azote dans un four à micro-ondes et la récupération du condensat à −80°C. Celui-ci a été extrait à l'hexane réduit à 100 μL et analysé par chromatographie en phase gazeuse. On a couramment décelé des concentrations aussi faibles que 5 ng de MIB par gramme de poisson. Dans la chair de poisson à mauvais goût, la bioconcentration de MIB s'élevait à 28,1 ± 14,0 (± ET) concentration dans l'eau/concentration dans le poisson. L'analyse de poissons à mauvais goût recueillis dans des usines de transformation a toujours révélé la présence de concentrations élevées de MIB. La géosmine, un autre composé qui entraîne des problèmes de saveur et d'odeur dans la chair du poisson, n'a pas été notée.

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Musty, earthy, and muddy are all descriptors of an odor and flavor problem that affects municipal water supplies (Izaguirre et al. 1982; Tabachek and Yurkowski 1976) and natural (Kuusi and Suihko 1983) and cultivated fish populations (Yurkowski and Tabachek 1980). Geosmin and 2-methylisoborneol (MIB) are the two primary chemical compounds implicated in this musty odor and flavor. Both compounds are tertiary alcohol secondary metabolites of some species of Cyanobacteria and actinomycetes (Gerber 1983; Izaguirre et al. 1983). When these compounds are absorbed by fish from the water, they may impart an objectionable taste and odor to the fish flesh. Absorption of these compounds from water by fish is thought to occur primarily through the gills, with skin and gut absorption of lesser importance (Lovell and Sackey 1973; From and Hrlyck 1984).

Odor problems in fish and water are most prominent when eutrophic water conditions are prevalent (Sivonen 1982; Persson 1982; Juttner 1984; Brown and Boyd 1982). High feeding rates and high stocking densities combine to produce a eutrophic environment and result in off-flavor in about one half of the ponds used in the commercial culture of catfish. During these off-flavor episodes, which occur when water temperatures are high, fish flesh becomes unpalatable and thus unmarketable. Commercial catfish producers are forced to keep and feed market weight fish until they lose their off-flavor.

Historically, organoleptic analysis has been utilized to detect these odorous compounds in water and fish flesh. Recent developments in analytical methods for water analysis have enabled researchers to approach the sensitivity of the human nose in detecting musty odorants (Onodera et al. 1983; Hwang et al. 1984; Veijanen et al. 1983; Lalezary et al. 1984). The threshold odor concentration (TOC) in water has been mea-

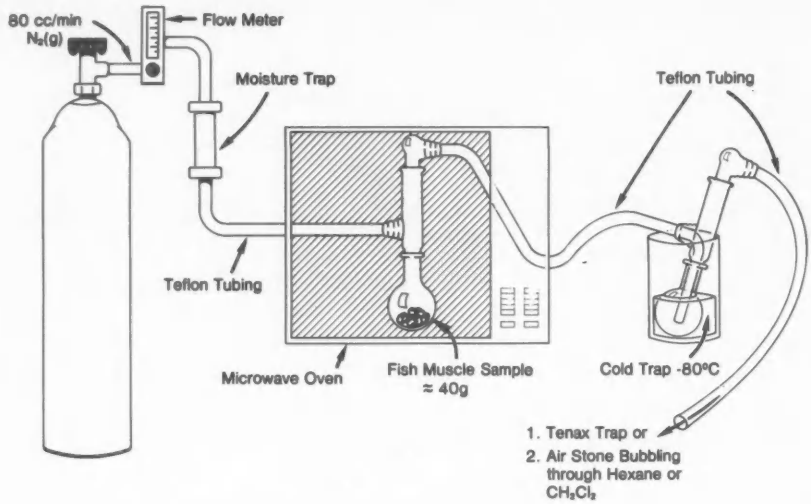

FIG. 1. Microwave cooking appartus for the rapid isolation of volatiles from fish flesh.

TABLE 1. Summary of analysis of water, mud, and fish of musty off-flavor ponds. Concentration of MIB (ng·g sample^{-1}) in the flesh of channel catfish, and water from commercial culture ponds of Mississippi.[a]

Pond No. - date of collection	Mud	Water	Fish	*n* (range)
8-8/13	14.9	10.06	284.0	5 (87–449)
8-8/27	8.46	2.14	90.2	1
8-9/10	1.00	0.10	24.9	1
8-9/24	1.00	0.10	14.4	2 (13.8–15.0)
10-8/27	2.71	13.32	187.1	3 (142–229)
10-9/24	1.00	0.10	5.0	2 (5.0–5.0)
10-10/8	1.00	0.10	18.6	3 (15.7–23.1
13-7/11	2.30	0.29	84.8	2 (83.4–86.1)
13-7/25	1.71	10.41	10.4[b]	3 (<5, <5, and 10.4)

[a]On other ponds, 9, 11 and 12, MIB in water, mud, and flesh of catfish was not measurable from April 30 to October 22, 1985, and fish were not off-flavored organoloptically.

[b]Only detectable concentration among the three fish analyzed.

sured at 0.02–0.04 $\mu g \cdot L^{-1}$ for geosmin and MIB. Analytical methods are now available that will isolate these odorants from water at subnanogram per litre concentrations (Onodera et al. 1983; Hwang et al. 1984). Analytical methods to isolate and quantify these compounds from fish flesh are not as well developed.

In this report, we describe a method for the isolation and quantification of MIB in fish flesh at the microgram per litre level. We also compare the concentrations of MIB in water and mud with those in fish flesh obtained during three documented off-flavor episodes.

Materials and Methods

Channel catfish (*Ictalurus punctatus*) (400–800 g) were obtained from commercial catfish culture ponds (Farm fresh Inc., Indianola, MS, ponds 8–13) and from a fish processing plant (Delta Pride Inc., Indianola, MS). Mud, water, and fish samples were collected from fish ponds every 2 wk from April 30, 1985, through October 22, 1985. The mud and water were examined for five musty odorants using closed loop stripping (Grob and Zurcher 1976). Off-flavored channel catfish samples were also collected directly from the processing plant. At the processing plant, the taste panel tested all fish for the presence of off-flavor. The whole channel catfish were transported on ice to the laboratory, eviscerated, transversely sliced into 1-cm-thick sections, and kept frozen at −80°C until analysis.

TABLE 2. Results from analysis of fish determined off-flavor organoleptically.

Date of collection - fish	Concentration of MIB (ng·g^{-1})	Fish flesh cooking conditions
7/25-1	103.9 ± 15.2	Hexane extract
7/25-2	24.2 ± 2.6	Hexane extract
7/11-1	392.6 ± 46.4	Cooked with hexane
7/11-2	5.0 ± 1.4	Cooked with hexane
8/13-1	815.5 ± 91.9	Cooked with hexane
8/13-2	247.8 ± 42.3	Cooked with hexane
8/13-3[b]	189.9 ± 22.7	Cooked with hexane
8/13-3[b]	210.5 ± 24.8	Hexane extract
8/27-1	18.0 ± 3.1	Cooked with hexane
8/27-2	344.0 ± 20.3	Cooked with hexane
8/27-3	69.2 ± 7.4	Cooked with hexane
10/8-1	6.3 ± 1.8	Hexane extract
10/8-2	38.2 ± 3.1	Cooked with hexane

[a]Samples collected from Delta Pride Inc., Indianola, MS. Each fish was cooked twice.

[b]Successive analyses of the same fish run twice by each method.

The following method was used in the preparation and analysis of off-flavored fish samples. About 40 g of fish muscle was cut into approximately 1-cm squares and placed in a dry 100-mL boiling flask with a 24/40 ground glass fitting (Fig. 1). Reagant grade hexane (J. T. Baker Chemical Co., Phillipsburg, NJ) (1 mL·5 g fish^{-1}) was added to some samples prior to cooking. The boiling flask was fitted with adaptors (Kontes, Vineland, NJ) and equipped with side-arm tapered

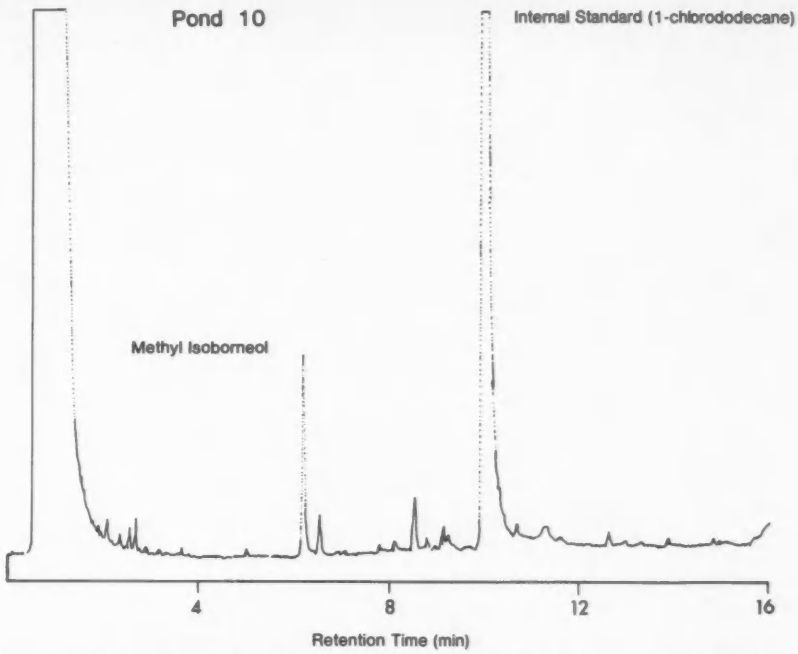

FIG. 2. Gas chromatogram of volatiles isolated from fish flesh using the microwave cooking method.

glass fittings into which 1/16-in. O.D. teflon tubing was inserted. The apparatus was continuously purged with nitrogen at 80 $cm^3 \cdot min^{-1}$ during cooking. The distillate was frozen by immersing a system of glassware identical to the cooking apparatus in a dewar filled with acetone cooled with dry ice (−80°C). An airstone connected to the cold trap was immersed in a hexane solution to collect volatiles that were not collected by the cold trap.

The fish (or fish plus hexane) was cooked in a microwave (Litton Generation II, Litton Systems, Inc., Minneapolis, MN) using 15-s bursts at 40% full power (580 W) for a total cooking time of about 3.5 min. After cooking, the hexane condensate or extract (depending on whether the fish were cooked or extracted with hexane) was concentrated to approximately 100–200 μL in a Kuderna Danish-concentrator and analyzed by gas chromatography.

The gas chromatograph (Perkin Elmer Sigma 3) equipped with flame-ionization detector and capillary DB-5 column (J & W, 0.32 mm I.D., 30 m long) was operated under the following conditions: injector, 275°C; detector, 325°C; initial temperature, 40°C; initial hold, 1 min; ramp rate, 8°C·min^{-1}; final temperature, 275°C; split ratio, 3:1. Five musty standards were 2-isopropyl methoxypyrazine (97% pure) (Aldrich Chemical Co. Inc., Milwaukee, WI), 2-isobutyl-3-methoxy pyrazine (70% pure) (Aldrich), 2-isobutyl-6-methoxypyrazine (30% pure) (Aldrich), MIB (Environmental Protection Agency (EPA), Cincinnatti, OH), and geosmin (EPA). The internal standard used was 1-chlorododecane (Aldrich). Gas chromatographic mass spectroscopic analyses were done with a Finnigan 4510 mass spectrometer and (Varian 3500) gas chromatograph equipped with a capillary DB-5 column (J & W, 0.32 mm I.D., 30 m long). Temperature programming conditions were hold 1 min at 40°C, increase to 100°C at 40°C·min^{-1}, then to 250°C at 10°C·min^{-1}, and hold at 250°C for 5 min.

Results and Discussion

The commercially raised off-flavored channel catfish flesh contained high levels of MIB, which was reflected by the high concentrations of MIB in mud and water from the rearing ponds (Table 1). A gas chromatogram of the extract and mass spectrum of the MIB peak (with a relative retention time of 5.78–5.81 min) isolated from flesh of pond 10 catfish are presented in Fig. 2 and 3, respectively.

Analyses were also done on off-flavored fish obtained from a commercial fish processing plant. These fish had been tested organoleptically and were off-flavor. About 80% (12 of 15 analyzed) of the catfish had high concentrations of MIB in their flesh (Table 2). The highest MIB concentration found was 812 ± 46 (±SD) ng·g^{-1}.

In channel catfish with organoleptically acceptable flavor, the levels of MIB, geosmin, or the three pyrazine isomers could not be measured by our method. However, when flesh with acceptable flavor was spiked with MIB, 62 ± 6% of MIB was recovered when cooked with hexane and 59 ± 8% when the condensate was extracted with hexane after cooking. When distilled water spiked with MIB was either cooked with hexane or the condensate extracted with hexane, similar recoveries were obtained (58 ± 11%). A single hexane extraction recovered 77 ± 5% of MIB in an aqueous solution.

The microwave method was tested for reproducibility. When the analysis of three different fish were replicated five times, the concentrations of MIB in each fish were 87.3 ± 8.6, 18.2 ± 3.1, and 181.4 ± 10.8 ng·g^{-1}. Conversely, different fish collected on the same day from the same pond

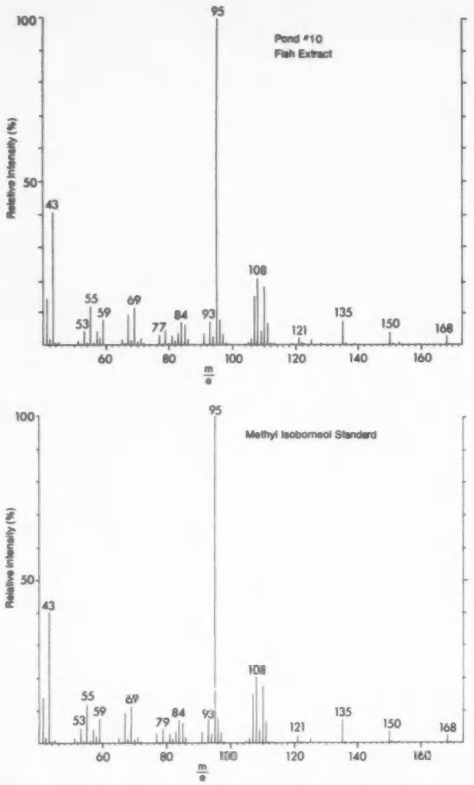

FIG. 3. Mass spectra of peak in Fig. 2 isolated from off-flavor fish obtained from pond 10 identical to the mass spectra of the MIB standard.

often showed markedly different levels of MIB in their flesh (Table 1, pond 8 collected August 13 (8-8/13)). Therefore, the microwave method gave acceptable recoveries (approximately 60%) and reproducible results with the same fish. The recovery of MIB by this method in off-flavored fish is probably similar to fish and water samples spiked with MIB. Reported values in off-flavored fish have been corrected for the loss of MIB attributable to extraction efficiency and concentration of the hexane extract of MIB.

The results show that channel catfish have higher levels of MIB than are present in water and mud; thus, MIB concentrations are variably magnified in the flesh (Table 1). Ratios of fish to either mud or water concentrations result in a wide range of bioconcentration values. During an off-flavor episode, the concentration of MIB in the mud and water may rise and fall rapidly within 2 wk whereas the fish retain the compound in their flesh for 2–6 wk after the concentration of MIB in the mud and water has dropped below detectable concentrations. In addition, results show that variations in the concentrations of MIB occur in different fish from the same off-flavored pond collected on the same day (Table 1). Comparing MIB concentrations of fish with those of water and mud (using values >1 ng·g^{-1}) from ponds 8-8/13, 8-8/27, 10-8/27 and 13-7/11 (mud only), the biomagnification of MIB concentration in fish flesh was 33.9 ± 25.6 and 28.1 ± 14.0 times that in mud and water, respectively. This biomagnification in flesh may be attributed to the lipophilic nature of MIB. The differences in biomagnification of MIB may be due to variations in metabolic activity, body fat content, age, nutrition status, or other factors. Further studies are necessary to substantiate the factors causing this biomagnification phenomenon.

Using our method of analysis, we have showed consistently the presence of elevated levels of MIB in off-flavored catfish from the Mississippi delta region. The results show that the musty, muddy, odor problem in these channel catfish occurs during the summer months and is primarily due to MIB. Other musty odorants (geosmin and the pyrazine isomers) were not detected. Microwave cooking, trapping the condensate at −80°C, and analyzing the hexane extract by gas chromatography possesses several advantages over vacuum distillation. These advantages include reproducibility, sensitivity at the nanogram per gram level, and reduced time of analysis.

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PERSPECTIVES

An Alternative Perspective on Recruitment Overfishing and Biological Reference Points

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Sissenwine, M. P., and J. G. Shepherd. 1987. An alternative perspective on recruitment overfishing and biological reference points. Can. J. Fish. Aquat. Sci. 44: 913–918.

Biological reference points are used to guide fisheries management decisions. The reference points most often used are expressed in terms of fishing mortality rate (F). F_{msy} relates to the maximization of sustainable yield. In principle, it is a most useful reference point, but in practice it is difficult to estimate. F_{max} and $F_{0.1}$ relate to certain levels of yield per recruit and are easily estimated, but they ignore conservation of the resource. Recruitment overfishing has usually been understood to occur when a population has been fished down to a point where recruitment is substantially reduced or fails. It has not been used as a basis for a biological reference point because the definition is vague and cannot be readily related to fishing mortality. Levels of spawning biomass below which recruitment seems to be reduced have been used, but their determination from available data is usually difficult and controversial. We propose an alternative definition of recruitment overfishing in terms of the level of fishing pressure that reduces the spawning biomass of a year class over its lifetime below the spawning biomass of its parents on average. Conventional models and types of data can be used to determine this level of F, denoted as F_{rep}, which clearly relates to the replacement of spawning biomass and thus to sustainability of a population and yield in the long term.

Les gestionnaires utilisent des points de référence biologique pour prendre des décisions sur la gestion des pêches. Les points de référence les plus souvent utilisés sont exprimés en taux de mortalité par pêche (F). Le F_{rms} exprime la maximalisation du rendement soutenu. En principe, c'est un point de référence très utile mais en pratique, il est difficile à estimer. Les F_{max} et $F_{0.1}$ expriment certains niveaux de rendement par recrue et sont facilement déterminés quoiqu'ils ne tiennent pas compte de la conservation de la ressource. Une surpêche au détriment du recrutement a généralement lieu quand une population est exploitée jusqu'au point où le recrutement est nettement réduit ou inexistant. Ce facteur n'a pas été utilisé comme base pour un point de référence biologique parce que sa définition est vague et qu'il ne peut pas être facilement transformé en niveau de mortalité par pêche. On s'est servi des niveaux de la biomasse génitrice au-dessous desquels le recrutement semble être réduit, mais la détermination de tels niveaux à partir des données disponibles est généralement difficile et sujet à controverse. Les auteurs proposent une autre définition de la surpêche au détriment du recrutement comme le niveau de pression par pêche qui réduit la biomasse génitrice d'une classe d'âge au cours de son cycle vital à un niveau inférieur à la biomasse génitrice parentale moyenne. Les modèles et données ordinaires peuvent être utilisés pour déterminer ce niveau de F, représenté par F_{rep}, qui explique clairement le remplacement de la biomasse génitrice et donc la permanence d'une population et le rendement à long terme.

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The terms "recruitment overfishing" and "biological reference points" are familiar to fishery scientists concerned with the conservation and management of fishery resources. In fact, they are related to each other although it is rare that the relationship is made explicit. The purpose of this paper is to offer an alternative perspective on recruitment overfishing and biological reference points. This is achieved by redefining recruitment overfishing.

Fisheries are managed to achieve certain socioeconomic benefits while conserving the production potential of the fishery resource. Since the goals of fisheries management are usually complex and difficult to quantify, it is necessary to establish operational objectives to guide the decision process. These operational objectives (referred to as proximate goals by Pope (1983)) usually address conservation and yield implications of fisheries management explicitly, but not economic and social considerations. They are referred to as biological reference points by institutions (e.g. International Council for the Exploration of the Sea (ICES)) concerned with fisheries management.

It would be useful to have biological reference points which take account of recruitment overfishing. The concept of recruitment overfishing was introduced by Petersen (1894) according to Cushing (1976). Yet, its definition is vague. Recruitment overfishing is usually understood to occur when a population has been fished down to a point where recruitment is substantially reduced, or perhaps fails altogether. Unfortunately, the definition places the emphasis on the results of overfishing instead of on the act of fishing. It presumes a compensatory relationship between recruitment and spawning biomass.

Traditional Derivation of Biological Reference Points

Biological reference points are derived from models characterizing fish population dynamics. The most frequently applied models are of three types: spawner–recruit, dynamic pool, and production models.

Spawner–recruit models describe the relationship between generations (e.g. Ricker 1954; Beverton and Holt 1957). Most models assume that the per capita recruitment rate (R/S) decreases as S increases, i.e. compensation. Compensation, during at least one life stage, is necessary if there is to be a sustained yield.

Dynamic pool models (e.g. Beverton and Holt 1957; Thompson and Bell 1934) describe the change in year class (or cohort of fish of the same age) biomass following recruitment. The number of individuals in the year class is reduced by fishing and natural mortality. The biomass of the year class is the product of its size in numbers and the mean weight of individuals. In general, unexploited or lightly exploited year classes increase in biomass following recruitment (when growth rate is relatively high) to a peak, and then biomass declines (as growth rate moderates). The yield of a year class is determined by the age at which fishing begins (t_c) and fishing mortality rate (F). If fishing begins too soon or if the fishing mortality rate is too high, then the fish are harvested before the year class has had a chance to achieve its maximum biomass and some potential yield is lost, and vice versa.

Fishing also affects the spawning biomass produced by a year class over its lifetime. The lifetime spawning biomass of a year class is the sum (over ages, indexed by i) of the biomass at each age (B_i) times the fraction mature at age (x_i), $\Sigma_i\, x_i B_i$. Fishing pressure (F and t_c) reduces lifetime spawning biomass of a year class.

Yield and spawning biomass are usually normalized to the number of recruits. The methods are also known as yield per recruit (Y/R) analysis or spawning biomass per recruit (S/R) analysis.

The production of a population (in biomass) is the sum of the weight of recruitment and the weight gain (i.e. growth) of individuals in the population minus the weight loss due to natural mortality. Production models (e.g. Schaefer 1957) describe production as a function of biomass, or equilibrium production as a function of fishing pressure. They are usually heuristic models of the aggregate effect of recruitment, growth, and natural mortality. They assume that per capita production decreases as biomass increases or fishing pressure decreases, i.e. compensation. Production models need not be heuristic. They can be derived by combining a spawner–recruit model and dynamic pool model (Shepherd 1982).

A graphic representation of spawner–recruit, dynamic pool, and production models is given in Fig. 1. The control variables are F and t_c. Together they characterize the intensity of fishing pressure. Superimposed on the graph of the spawner–recruit function is a line with a slope equal to the inverse of S/R from the dynamic pool model. This line is referred to as a "replacement line." Its intersection with the spawner–recruit function determines the equilibrium values of S and R for a specific level of fishing pressure. The corresponding equilibrium production is the product of the equilibrium R and the Y/R. By varying fishing pressure, a production model can be constructed.

Several biological reference points are defined in Fig. 1. F_{max} is the fishing mortality rate that maximizes yield per recruit. While F_{max} has obvious merit relative to short-term yield (for recruits already vulnerable to the fishery), it ignores the effects of fishing on future generations. $F_{0.1}$ is often proposed as an alternative, since it produces nearly as much yield without reducing spawning biomass so severely. The yield per unit of fishing mortality is higher as well. $F_{0.1}$ is defined as the fishing mortality rate that corresponds to a point on the yield per recruit function with a slope of 10% of the slope through the origin (Gulland and Boerema 1973). While the effect of fishing on the future recruitment is one of the considerations behind $F_{0.1}$, the relationship is speculative at best.

Biological reference points based on a production model (Fig. 1e) incorporate the effect of fishing on future generations. MSY is a biological reference point specified in terms of yield. It corresponds to a fishing mortality of F_{msy} at equilibrium. A biological reference point in terms of equilibrium biomass can also be specified.

Recruitment overfishing is usually understood to occur when spawning biomass is so low that recruitment decreases substantially and perhaps precipitously. While this vague definition does not lend itself to exact specification of a biological reference point, a fishing mortality rate of F_{ro} and the corresponding spawning biomass of S_{ro} in Fig. 1 are good candidates. The specific value labeled as F_{ro} is arbitrary, but it is clear that an increase in fishing mortality will result in a sharp reduction in equilibrium recruitment. A more extreme choice of a biological reference point would be the level of fishing mortality that corresponds to a replacement line that is so steep that it only intersects the spawner–recruit function at the origin. This implies that the population will be fished to extinction. In theory, a population can persist with a very low level of equilibrium recruitment so long as F corresponds to a replacement line with slope less than the slope of the spawner–recruit function at the origin. If a spawner–recruit function is known, the definition of recruitment overfishing can be quantified in terms of the slope of the function, e.g. fishing mortality rate that corresponds to an equilibrium point on the spawner– recruit function with slope of 10% of the slope at the origin.

Based on the theory, there are ample choices of biological reference points, including some that correspond in some way to recruitment overfishing. But to apply the theory, there must be sufficient information to estimate a compensatory spawner–recruit function. More often than not, the data provide little support for the models (see review by Sissenwine et al. 1984). It should be apparent from Fig. 1 that unless a spawner–recruit model can be fitted to the data with a reasonable level of confidence, then only $F_{0.1}$ and F_{max} are available as biological reference points. In fact, that is why they are applied most often.

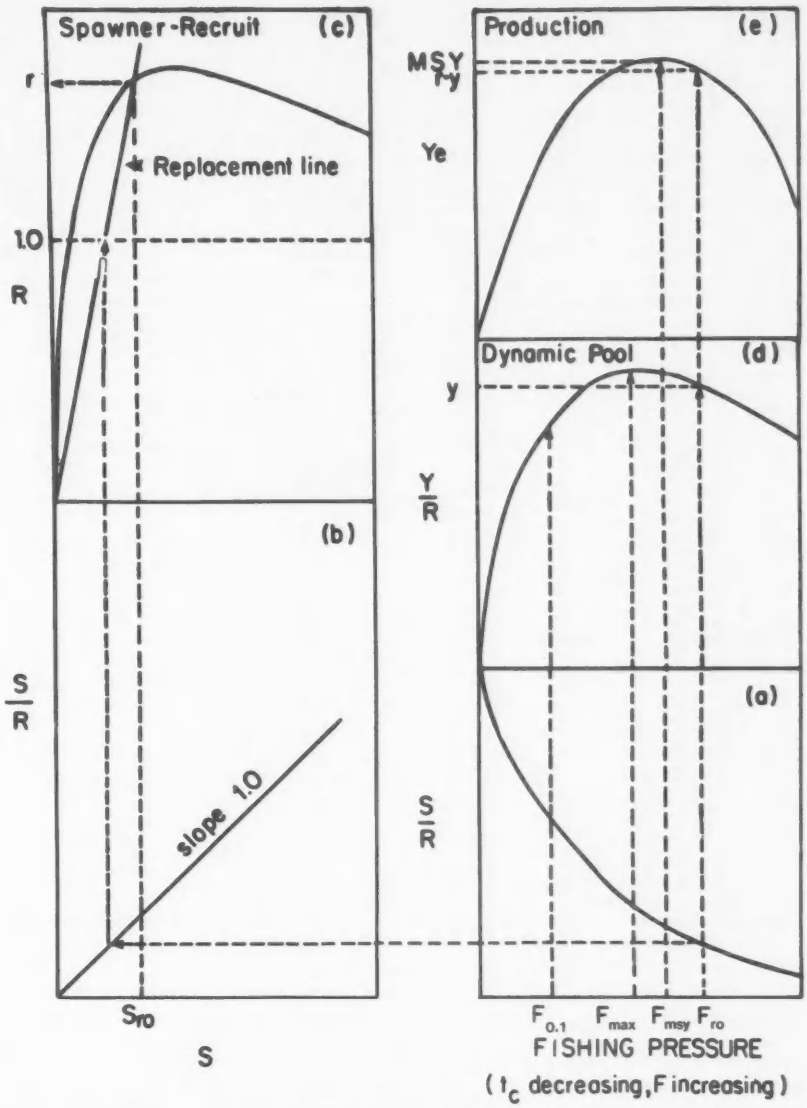

FIG. 1. Single species theory of fishing. A dynamic pool model (Fig. 1a and 1d) describes the effect of fishing mortality rate (F) and age at first capture (t_c) on spawning biomass (S) per recruit (R) and yield (Y) per recruit. A spawner–recruit model (Fig. 1c) relates the number of recruits to spawning biomass; a "replacement line" with slope equal to the inverse of S/R is mapped by a graphic procedure (Fig. 1b). S and R must be scaled to appropriate units (e.g. thousands of tons and millions of fish, respectively) in order for the graphic procedure to be practical. The intersection of the replacement line with the spawner–recruit function determines equilibrium recruitment. The product of equilibrium recruitment (from Fig. 1c) and yield per recruit (from Fig. 1d) is the equilibrium production (Fig. 1e). The method is demonstrated for a fishing mortality rate of F_{ro} (conditional on an unspecified value of t_c). F_{msy} is indicated based on the production model of Fig. 1c; $F_{0.1}$ and F_{max} are indicated in Fig. 1d.

There are three explanations for the poor fit of spawner–recruit models: (1) the models may be too simple biologically, (2) variability caused by abiotic factors obscures biotic relationships, and (3) measurement error (Walters and Ludwig 1981). Undoubtedly, all three contribute to the problem.

It can be argued that S_{ro}, MSY, and F_{msy} can be estimated without a spawner–recruit model. One argument is that it is not necessary to be able to fit a compensatory spawner–recruit model in order to detect a level of S below which the magnitude of R declines precipitously. But the formal statistical interpretation of not being able to fit a compensatory spawner–recruit model to a data set is that the null hypothesis

of a constant per capita recruitment rate (R/S) cannot be rejected. R is lower for low values of S, but the data do not usually indicate a specific level of S where a marked change in the pattern occurs. The traditional concept of recruitment overfishing implies compensation; if the hypothesis of compensation cannot be supported by the data, then there is no basis for a biological reference point corresponding to recruitment overfishing.

Another argument is that a production model can be fitted to catch and fishing effort data directly without considering the spawner–recruit relationship. Therefore, it may be possible to estimate MSY and F_{msy} from a production model even if the spawner–recruit model does not fit the available data.

But why should a heuristic production model which aggregates recruitment with growth and natural mortality be able to explain the dynamics of a fishery if the spawner–recruit data are so variable that a relationship cannot be fitted? Even though the spawner–recruit relationship is not treated explicitly in the heuristic production model, it is still a component of production. It is frequently the largest component (e.g. southern New England yellowtail flounder; Sissenwine 1974) and the component where compensation is usually assumed to occur.

In fact, there are more examples of the "successful" application of production models than spawner–recruit models. In part, this is because the data used to fit the former are more often available than the data to fit the latter. But, we wonder if some of the apparent success of production models might reflect the frequent use of an inappropriate method for fitting. The procedure for fitting production models is often simplified by assuming that data are drawn from a fishery at equilibrium. The assumption is virtually always invalid. Even if it were not, the linearized equilibrium version of the most frequently applied production model (i.e. Schaefer, 1957) may indicate a spurious fit to data (even random numbers) when none exists (Knights and Pope, unpublished, see Sissenwine 1978; Kenney 1982). There are methods for fitting production models (Rivard and Bledsoe 1978) that do not suffer from this problem, but their application is not as common. It should be noted that the same type of spurious correlation that can result when fitting linearized equilibrium production models can result from the linearized version of some spawner–recruit models.

An Alternative Approach when the Spawner–Recruit Relationship is Indeterminate

The spawner–recruit relationship is indeterminate when there is no statistical evidence of a trend in R/S over the observed range of S. But, the observed values of R/S can be used as a basis of an alternative definition of recruitment overfishing and a biological reference point.

A priori, persistence of a population requires that each year class replace the spawning biomass of its parents on average. We have already shown that a dynamic pool model can be used to calculate the effect of fishing on the lifetime spawning biomass, and that these values of S/R correspond to replacement lines on a spawner–recruit plot (i.e. $L = S/R = H(F, t_c)$. Data points that lie on a replacement ($R/S = L^{-1}$) line correspond to year classes that are the exact size to replace the spawning biomass of their parents when subjected to a level of fishing pressure that corresponds to the line. Points above the replacement line ($R/S > L^{-1}$) are year classes that produce a surplus spawning biomass; points below the line ($R/S < L^{-1}$) are year classes that do not produce enough. For a population to persist, the points below the line must be balanced by the points above it.

In this context, we define recruitment overfishing as a level of fishing pressure that reduces the spawning biomass produced by a year class over its lifetime below the spawning biomass of its parents on average, i.e. recruitment overfishing corresponds to $L^{-1} > E(R/S)$ which is the expected value of R/S. We denote the fishing mortality rate that corresponds to average replacement as F_{rep} (conditional on t_c). F_{rep} is a useful biological reference point. If F exceeds F_{rep} for an extended period of time, population size will decline, and vice versa, assuming that the pattern of R/S does not change (i.e. assuming a stationary distribution).

This alternative definition of recruitment overfishing is illustrated for Georges Bank haddock in Fig. 2. The observed values of recruitment and spawning biomass are so variable that a spawner–recruit function was not fitted. Three replacement lines, corresponding to $F = 0.0$, 0.5, and 1.0, for $t_c = 2.0$ yr, are superimposed on the spawner–recruit data. Most of the points are below the replacement line for $F = 1.0$, indicating that the population will decline in size, on average, at this level of fishing mortality. On the other hand, the figure indicates that the population will grow if fishing ended, but it is noteworthy that some year classes are too small to replace their parents even with no fishing. The replacement line of $F = 0.5$ is the median (i.e. equal number of points above and below). For a value of $F = 0.5$, the population will fluctuate in size without trend.

For this illustration, we estimate F_{rep} as 0.5. It is approximately equal to F_{max} which is about 0.55 and much higher than $F_{0.1}$ which is about 0.25. Technically, MSY and F_{msy} are indeterminate because there is no statistical evidence of compensation. Nevertheless, it is possible to get some idea of the outlook for long-term average yield (quasi-MSY) from Fig. 2. The average recruitment, over the observed range of spawning biomass, is about 50 million fish. The product of the average recruitment and the Y/R corresponding to F_{rep} (about 1 kg) is about 50 000 tons. In fact, the population sustained an average catch of about 50 000 tons for 30 yr when it was fished at a fishing mortality rate of about 0.5. When the fishing mortality rate increased in the mid-1960's, the population collapsed (Clark et al. 1982). So, for the Georges Bank haddock, it appears that F_{rep}, F_{max}, and F_{msy} are about the same value. Recruitment overfishing occurs when F is greater than 0.5. Since the data do not support the hypothesis of a compensatory spawner–recruit function, a critical value of spawning biomass that corresponds to recruitment overfishing cannot be specified.

Discussion

This paper provides an alternative perspective on the interpretation of spawner–recruit data and dynamic pool models when the spawner–recruit relationship is indeterminate; it is not an argument against compensation in fish populations. Compensation must exist at some level of population size, since life-sustaining resources (e.g. food, space) are limited. Compensation need not be operative over the range of spawning biomass for which there are quantitative observations. For the population to persist where compensation is inoperative, F should not exceed F_{rep}. If compensation is operative, but

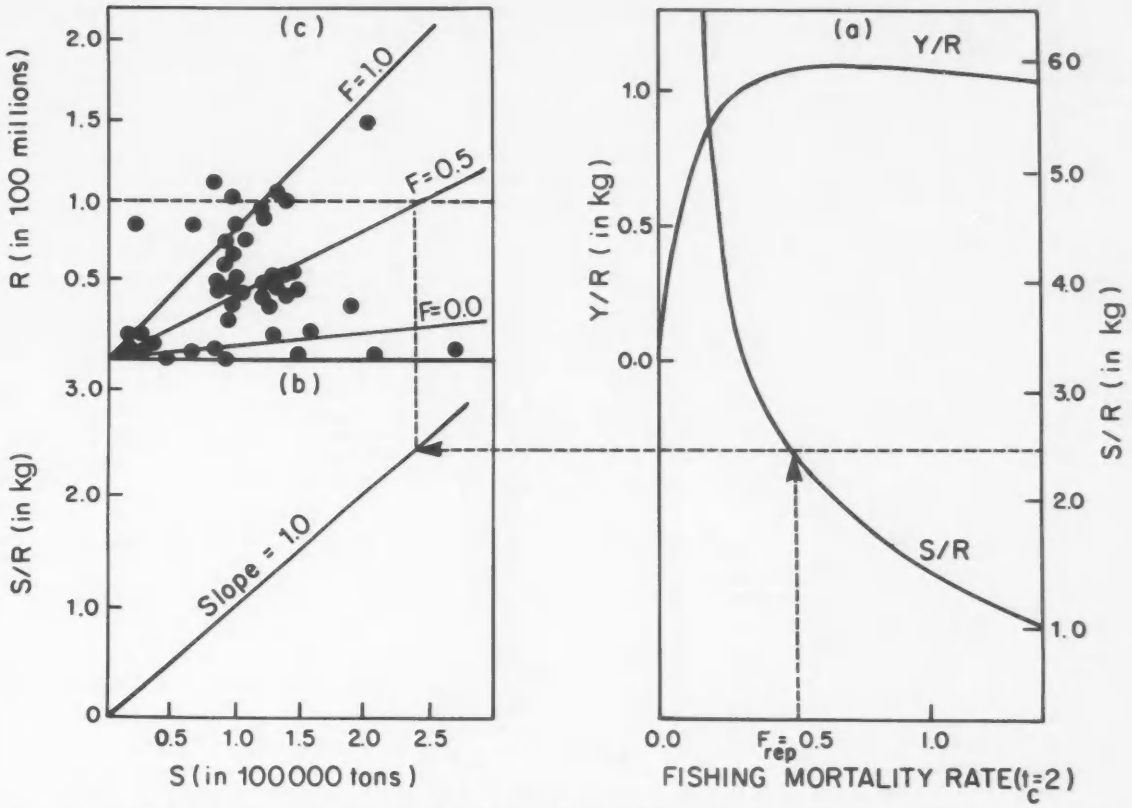

FIG. 2. Population dynamics of Georges Bank haddock (after Sissenwine and Marchesseault 1985; Sissenwine et al. 1984). One data point (S = 1.94 and R = 3.68) has been omitted. Replacement lines corresponding to several levels of fishing mortality (with t_c = 2.0 yr) are derived from the spawning biomass per recruit analysis (Fig. 2a) and superimposed on the spawner–recruit plot (Fig. 2c). For specific level of fishing pressure, points above the replacement line represent year classes that will produce more than enough lifetime spawning biomass to replace their parents; points below will not. The replacement line for F = 0.5 is the median, e.g. an equal number of points above and below. If the median is taken as an estimate of the mean, then a fishing mortality rate of 0.5 will allow year classes to replace the spawning biomass of their parents on average. A fishing mortality rate that corresponds to replacement on average is denoted by F_{rep}.

obscured by the effects of a fluctuating environment, then F_{rep} is a conservative biological reference point. This is appropriate in the absence of statistical evidence that can be used to quantify compensation.

Since compensation must be operative at some high level of biomass, F_{rep} is most likely to be conservative for a short data set drawn from a developing fishery. In such cases, it will be necessary to apply an adaptive management strategy in order to discover the nature of the spawner–recruit relationship, i.e. either to fit a spawner–recruit model or to be convinced that F_{rep} is not conservative. If an estimate of F_{rep} is greater than or equal to F_{max}, it is not conservative in terms of yield, if the spawner–recruit relationship is asymptotic. If the spawner–recruit function is dome-shaped, $F_{rep} \geq F_{max}$ may be conservative although we think this is unlikely.

There are several limitations to the approach described herein. First, the method assumes that the observations of S and R are from a stationary distribution, i.e. past events are a useful basis for forming expectations about the future. This assumption can be investigated by examining the data to determine if R/S has a temporal trend. If a trend is suspected, then the most recent observation should be weighted more heavily.

Second, the approach requires data describing S and R. These data are not available for all fisheries, although they are for a large number. If S and R data are missing, F_{rep} can be inferred from a period during which a fishery was relatively stable. In the case of Georges Bank haddock, observations from a relatively stable period were used to confirm an estimate of F_{rep}.

Third, the approach does not consider the potential effects of depensation, i.e. R/S decreasing as S decreases. If there is depensation, then F_{rep} is not a conservative biological reference point. This problem can be alleviated to some degree by weighting the most recent values of R/S more heavily. In effect, this narrows the range of S over which the mean of R/S is calculated.

Fourth, it requires estimates of biological parameters such as age at maturity (t_m), natural mortality rate (M), and growth rate or size at age (W_t) to calculate S/R from a dynamic pool model. These parameters are also required to estimate R and S by the most popular method (virtual population analysis (VPA), Murphy 1965). If the same values of t_m, M, and W_t are

TABLE 1. Effect of overestimating age at maturity (t_m), natural morality (M), and weight at age (W_t) on dynamic pool model results and spawner–recruit plots when S and R are derived by virtual population analysis (VPA). Underestimates are indicated by a minus sign, overestimates are indicated by a plus sign, a greater degree of overestimation is indicated by two plus signs, no effect is indicated by zero.

Overestimated parameter	Dynamic pool		Spawner–recruit		
	$L = S/R$	L^{-1}	R	S	R/S
t_m (+)	−	+	0	−	+
M (+)	−	+	++	+	+
W_t (+)	+	−	0	+	−

used to calculate L^{-1} by a dynamic pool model and to estimate R and S by VPA, then estimates of F_{rep} should be robust. Table 1 indicates the effect of overestimating t_m, M, and W_t on L^{-1} from a dynamic pool model and R/S (derived by VPA) on a spawner–recruit plot. In each case, the errors are in the same direction so that the relative position of replacement lines and points on a spawner–recruit plot are not sensitive to errors in the aforementioned biological parameters.

These limitations are not unique to this approach. They apply to the traditional definition of recruitment overfishing and of biological reference points. An advantage of our approach is that it is straightforward to define recruitment overfishing and biological reference points in terms of t_c as well as F. This flexibility is inherent in the coupling of a spawning biomass per recruit analysis (as a function of both F and t_c) and spawner–recruit data.

It is also possible to estimate the slope at the origin of the spawner–recruit plot to find a biological reference point (F_{high} in the terminology used by ICES 1984) more directly indicative of possible stock collapse. Shepherd (1982) suggested that using a value of F corresponding to the upper 90-percentile of the observed R/S values would be conservative because the population might be capable of more compensation at low stock levels than was evident in the data. Sissenwine et al. (1984) pointed out that this was not guaranteed because the effects of environmental variation would cause the observed extreme values of R/S to be inflated. There are, thus, two opposing effects, but what is certain is that there is little evidence in the data to support the idea that the population could support F values as high as F_{high} for any length of time. Thus, it may still serve as a useful indicator of dangerously high fishing mortality, as compared with F_{rep} which indicates a level which probably can be sustained indefinitely.

In this paper, the median is used to estimate the expected value of S/R as a basis to determine F_{rep}. One advantage of the median is that it is a robust estimator if S and R are measured with error. Other estimators should be considered.

The ideas in this paper have evolved over a few years. Shepherd (1982) reviewed the use of spawning biomass per recruit analysis to calculate production models and emphasized the connection between the slope at the origin and stock collapse. ICES (1984) introduced the idea of using spawning biomass per recruit as a basis of biological reference points. Shepherd and Sissenwine (see Beverton et al. 1984) expanded on the approach at a Dahlem Conference on "Exploitation of Marine Communities." At the same time, Sissenwine was working directly with fisheries managers to apply the approach (Sissenwine and Marchesseault 1985). The guideline of enough spawning biomass per recruit so that year classes replace the spawning biomass of their parents on average was adopted as an objective of the Multispecies Fisheries Management Plan for New England (NEFMC 1985). This paper was written to expose the approach more broadly to the scientific community.

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ANNOUNCEMENTS

Maurice Lamontagne Institute

The Maurice Lamontagne Institute, a new marine science research institute of the Department of Fisheries and Oceans, opened on December 1, 1986. Located on the shores of the St. Lawrence estuary in eastern Quebec near Rimouski, the Institute is one of the major Francophone centres for marine science research in the world.

The Institute is involved in all three of the Department's areas of scientific activity: fisheries research, oceanography, and hydrography. The purpose of its research programs is to generate the scientific information required for providing advice for the management, protection, and development of marine renewable and nonrenewable resources in fisheries, ocean industry development, commercial shipping, recreational boating, and the management of Quebec's coastal zones. Research activities focus primarily on the St. Lawrence estuary and on waters of northern Quebec, particularly James, Hudson, and Ungava bays.

By 1993 the Institute is expected to employ over 250 research scientists and technical and administrative support staff. It will also provide facilities for postdoctoral fellows, visiting scientists, and students.

Dependent upon the Institute are a satellite laboratory — the Arctic Biological Station at Sainte-Anne-de-Bellevue near Montreal — and field stations at Pointe-des-Monts, on the north shore of the estuary, and in northern Quebec.

Research teams from various units of the Institute cooperate in studying marine phenomena, and maintain close ties with universities and industry. Research results are disseminated through publications, lectures, and displays.

Institut Maurice-Lamontagne

L'Institut Maurice-Lamontagne, un nouvel institut de recherche en sciences de la mer du ministère des Pêches et des Océans, a ouvert ses portes le 1er décembre 1986. Situé dans l'est du Québec en bordure de l'estuaire du Saint-Laurent, près de Rimouski, l'Institut est l'un des principaux centres francophones du monde dans le domaine des sciences de la mer.

L'Institut regroupe les trois volets d'activités scientifiques du Ministère, soit la recherche sur les pêches, l'océanographie et l'hydrographie. Ses programmes de recherche visent à acquérir les informations scientifiques nécessaires à la formulation de conseils pour la gestion, la protection et la mise en valeur des ressources marines renouvelables et non renouvelables dans le domaine des pêches, pour l'exploitation sous-marine, la navigation commerciale et de plaisance et la gestion des zones côtières du Québec. Les travaux de recherche portent principalement sur l'estuaire du Saint-Laurent et sur les eaux du nord québécois, particulièrement les baies de James, d'Hudson et d'Ungava.

En 1993, on prévoit que l'Institut aura un effectif de 250 personnes, constitué de chercheurs scientifiques et du personnel de soutien technique et administratif. Il pourra également accueillir des chercheurs poursuivant des études post-doctorales, des scientifiques en visite et des étudiants.

L'Institut compte un laboratoire satellite, soit la Station de biologie de l'Arctique à Sainte-Anne-de-Bellevue près de Montréal, et des stations de terrain à Pointe-des-Monts, sur la rive nord de l'estuaire, et au Nouveau-Québec.

Les équipes de recherche de divers services de l'Institut collaborent à l'étude des phénomènes du milieu marin et entretiennent des relations étroites avec l'industrie et les universités. Les résultats de leurs recherches sont diffusés sous forme de publications, de conférences et d'expositions.

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